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(54) Title: COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE (57) Abstract Compositions that disrupt microvascular endothelial and epithelial cell tight junctions, and methods of use, are disclosed. Such compositions comprise agents that inhibit the binding to such cells of cell adhesion molecules. Such inhibitor agents include cell adhesion molecules, fragments of cell adhesion molecules that encompass a cell-binding domain such as HAV, and antibodies directed against cell adhesion molecules and fragments thereof. Also disclosed are drug delivery compositions comprising a therapeutic drug conjugated to an agent that disrupts cell tight junctions.		

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COMPOSITIONS FOR CELL ADHESION INHIBITION
AND METHODS OF USE

This is a continuation-in-part of United States
Serial No. 07/413,332, filed September 27, 1989.

5 Background of the Invention

Field of the Invention

 This invention relates to compositions that
transiently and reversibly dissociate the blood-brain
barrier. More particularly, the invention relates to
10 compositions that dissociate tight junctions between
brain capillary endothelial cells that constitute the
physiological barrier between the general circulation
and the brain.

Detailed Description of Related Art

15 The entry of drugs from the blood stream to the
central nervous system (CNS), i.e., the brain and
spinal cord, is restricted by the presence of high
resistance tight junctions between brain capillary
cells and by the apparently low rate of transport
20 across these endothelial cells (Betz, A.L., et al.,
Ann. Rev. Physiol., 48:241 (1986); Pardridge, W.M.,
Ann. Rev. Pharmacol. Toxicol., 28:25 (1988)).

 The tight junctions of the blood brain barrier
(BBB) prevent diffusion of molecules and ions around
25 the brain capillary endothelial cells. The only
substances that can readily pass from the luminal core
of the capillary to the abluminal tissues that surround
the capillary are those molecules for which selective
transport systems exist in the endothelial cells, as
30 well as those compounds that are lipophilic (i.e.,
hydrophobic). In contrast, drugs, peptides and other

molecules that are neither lipophilic nor transported by specific carrier proteins are barred from entry into the brain, or their rates of entry are too low to be useful, thereby imposing a severe limitation upon the physician's ability to treat CNS disorders pharmacologically.

The carrier-mediated transcellular transport system mentioned above may have limited usefulness for therapeutic modalities under some circumstances.

Transcytotic transport, in general, involves, first, the binding of molecules to specific carrier proteins on the surface of endothelial cells, and, second, the delivery of such molecules across the endothelial cells. Limitations on the usefulness of such a system for treatment of CNS disorders are based on the following considerations: (1) physiological carrier proteins may not function efficiently, or at all, with non-physiological drugs; (2) even where function occurs, the rate of transport of therapeutic agents will be limited by the rate of transport of the carrier; (3) the overall capacity of cerebral capillary endothelial cells to transport any therapeutic macromolecules may be simply too low to achieve therapeutic levels of certain drugs in the brain; and (4) once therapeutic macromolecules enter endothelial cells, depending on their nature, they might be delivered to any number of organelles, including lysosomes that contain a wide variety of hydrolytic enzymes. For these reasons, creating drug delivery systems that do not rely upon transcytosis will clearly be advantageous.

As tight junctions between brain capillary endothelial cells constitute a major part of the BBB, the possibility of modifying these junctions has been considered. It has been found that tight junctions,

including those of the BBB, can be disrupted by hyperosmotic solutions administered intra-arterially. For example, Polley et al., W089/04663, published June 1, 1989, disclose the osmotic disruption of the interendothelial structure of the BBB by the intra-arterial administration of hypertonic solutions of mannitol, arabinose or glycerol as a means of introducing into the brain genetic material. Similarly, hyperosmotic solutions of urea have also been used to alter the BBB (Bowman, P.D. et al., Ped. Res., 16:335A (1982)).

Other chemical agents have been reported to disrupt endothelial or epithelial cell tight junctions when administered intravenously, including:

7-fluorouracil (MacDonell, L.A., et al., Cancer. Res., 38:2930 (1978)), degradation by membrane enzymes (Vincent, P.A., et al., Exp. Mol. Path., 48:403 (1988)); Diener, H.M., et al., J. Immunol., 135:537 (1985)), aluminum salts (Zigler, Z.Y., et al., IRCS Med. Sci., 12:1095 (1984)), histamine (Meyrick, B., et al., Exp. Lung Res., 6:11 (1984)), thrombin (Siflinger-Birnboim, A., et al., Microvasc. Res., 36:216 (1988)), phorbol esters (Shiba, K., et al., Exp. Cell Res., 178:233 (1988)), and neutralization of the luminal anionic charge (Hart, M.M., J. Neuropathol. Exp. Neurol., 46:141 (1987)).

Although the above-listed modalities may disrupt tight junctions and thereby increase permeability of the BBB, problems attendant upon their use make them less than desirable. For example, intra-arterial perfusion with hyperosmotic solutions involves surgery, and this cannot be repeated on a regular basis. Further, concentrated sugar solutions may not be innocuous, and might be expected to have undesirable side effects. In addition, the aforementioned chemical

agents may not be useful for the treatment of chronic neurological disease, their effects on tight junctions are not always reversible, and, as they all are themselves powerful drugs, there is always the danger that their use will compromise the patient's health generally. For example, 7-fluorouracil is a powerful inhibitor of pyrimidine synthesis, and thus nucleic acid biosynthesis, in animals cells.

Thus, an important need still exists for means which transiently and reversibly disrupt tight junctions of the BBB in order that administered drugs can reach the brain from the general circulation, and which have no undesirable side effects of their own in the subject.

Attempts have been made to disrupt cell-cell adhesion by modifying the protein(s) responsible for such adhesion, collectively referred to as "cell adhesion molecules" (CAM). One class of CAM is termed "cadherin". "Cadherin" is the term applied to a family of glycoproteins found in most kinds of mammalian tissues and thought to be responsible for Ca^{2+} -dependent cell-cell adhesion, (Takeichi, M., Development, 102:639 (1988)). Three subclasses of cadherin have been identified, namely, E-cadherin (from epithelial tissues), P-cadherin (from placental tissues), and N-cadherin (from neural tissues) (Yoshida-Noro, C., et al. Dev. Biol., 101:19 (1984); Nose, A., et al., J. Cell Biol., 103:2649 (1986); Hatta, K., et al., Nature, 320:447 (1986)).

The different cadherins exhibit distinct tissue distribution patterns (Takeichi, U., (1988) above). E-cadherin, which was found to be distributed exclusively in epithelial cells of various tissues (Hatta, K., et al., Proc. Nat'l. Acad. Sci. (USA), 82:2789 (1985); Takeichi, 1988, above), appears to be

- identical to uvomorulin (Hyafil, F., et al., Cell, 21:927 (1986)), chicken liver-cell adhesion molecule (L-CAM, Gallin, W.J., et al., Proc. Nat. Acad. Sci. (USA), 80:1038 (1983)), and cell-CAM 120/80 (Damsky, C.H., et al., Cell, 34:455 (1983)) in terms of biochemical properties (Cunningham, B.A., et al., Proc. Nat. Acad. Sci. (USA), 81:5787 (1984)) and tissue distributions (Thiery, J.-P., et al., Dev. Biol., 102:61 (1984)).
- 10 N-cadherin, which is expressed in various neural tissues including astrocytes (Hatta, K., et al., Devel. Biol., 120:215 (1987); Matsunaga, M., et al., Nature, 334:62 (1988); Tomaselli, K.J., Neuron, 1:33 (1988)), shows 92% amino acid sequence homology between
- 15 mammalian and avian homologs, shows from 40 to 50% similarity to epithelial E-cadherin and to placental P-cadherin of the same species, but was immunologically not cross-reactive with other cadherins within the same animal (Miyatani, S., Science, 245:631 (1989)).
- 20 Placental P-cadherin has also been cloned, and the deduced amino acid sequence of this glycoprotein was found to exhibit about 58% homology with epithelial E-cadherin (Nose, A., et al., EMBO J., 12:3655 (1987)).
- Subsequent to the September 27, 1989 filing of the
- 25 parent application, Heimark, et al. (Heimark, R.L., et al., J. Cell Biol., 110:1745 (1990) reported on the identification of a Ca^{2+} -dependent cell-cell adhesion molecule in aortic endothelial cells.
- Although each of the aforelisted cadherins
- 30 displays unique immunological and tissue distribution specifications, all have features in common: (1) a requirement for Ca^{2+} for cell adhesion function; (2) protection by Ca^{2+} from proteolytic cleavage; (3) similar numbers of amino acids, i.e., from about 723 to
- 35 about 822; (4) similar masses, i.e., about 124 kdal.

for the glycoprotein; (5) substantial interspecies (50%-60%) overall sequence homology with interspecies homologies increasing to about 56% to 99% in the cytoplasmic region of the protein, suggesting that they constitute a gene family (Nose, A., 1987; Miysysni, D., et al., 1989); and (6) a common mechanism of action, namely, homophilic binding of cadherins on one cell to similar cadherins on the adjoining cell.

CAMs independent of Ca^{2+} are also known, for example, the 125K glycoprotein of Urushihara et al. (Urushihara, H., et al., Cell, 20:363 (1980)); N-CAM (Rutishauser, U., Nature, Lond., 310:549 (1984)); Ng-CAM (Grunet, M. et al., Proc. Nat'l. Acad. Sci. (USA), 81:7989 (1984)); L1 (Rathjien, F.G. et al., ***** J., 3:1 (1984)); G4 (Rathjien, F.G. et al., J. Cell Biol., 104:343 (1987)); and platelet glycoprotein PECAM-1 (CD 31) (Newman, P.J., Science, 247:1219 (1990)). Ca^{2+} -independent CAMs are known to exhibit certain properties of the Ca^{2+} -dependent CAMs. Thus, N-CAM and N-cadherin both promote retinal neurite outgrowth on astrocytes (Neugebauer, K.M., et al., J. Cell Biol., 107:1177 (1985)), and on Schwann cells (Bixby, J.L. et al., J. Cell Biol., 107:353 (1988)).

Monoclonal antibodies raised against epithelial E-type cadherins such as uvomorulin are known to disrupt the adhesion of several cell types, including embryo cells, cultured teratocarcinoma cells, hepatocytes, and MDCK kidney epithelial cells (Ogou, S.-I., et al., J. Cell Biol., 97:944 (1983); Yoshida-Noro, et al., (1984), above; Shirayoshi, Y., et al., Cell Struct. Funct., 11:285 (1986); Gallin, et al., (1983), above; Vestweber, D., et al., EMBO J., 4:3393 (1985); Johnson, M.H., et al., J. Embrol. Exp. Morphol., 93:239 (1986); Gumbiner, B., et al., J. Cell Biol., 102:457 (1986)).

However, prior to the present discoveries disclosed in the parent applications cadherins had not been found in brain capillary or other endothelial cells (see, Takeichi, et al. (1988), above). Further, the CAMs of microvascular endothelial cells had not yet been identified, nor had such molecules been localized specifically to brain capillary endothelial cells. Thus, until the present invention no means were known for transiently and reversibly disrupting tight junctions between microvascular endothelial cells, including those of the BBB, based upon an attack upon the CAM's of such cells that are responsible for tight junction formation and maintenance.

It has been hypothesized that the cadherins contain a common cell adhesion recognition (CAR) sequence. The CAR sequences of several cell and substratum adhesion molecules are known. Martin, G.R., et al., Ann. Rev. Cell Biol., 3:57 (1987) ; Ruoslahti, E., et al., Science, 238:491 (1987). In general, CAR sequences are composed of at least three amino acid residues. The most rigorously investigated CAR sequence is RGD which is found in laminin, fibronectin and other basement membrane components that are responsible for the binding of cells to the substratum.

Blaschuk, et al., in a paper to be published subsequent to the filing of the present application (Blaschuk, O., et al., J. Mol. Biol., in press, (1990)), disclose the presence of three potential cadherin CAR sequences in the first extracellular domains of liver CAM, E-, P-, and N-cadherin, namely, PPI, GAD and HAV. Blaschuk, et al. (Blaschuk, O., et al., Develop. Biol., 139:227 (1990)), also disclosed recently that synthetic peptides containing the HAV sequence inhibited two biological processes (compaction of 8-cell-stage mouse embryos and rate of neurite

outgrowth on astrocytes) that are known to be mediated by cadherins. Effective peptides in these assays were LRAHAVDVNG and AHAVSE; PPI-containing peptides were without effect. However, Blaschuk et al. provide no
5 guidance for determining the regions flanking the HAV tripeptide that are critical for cell-cell adhesion. In the BBB disrupting peptides of the present invention detailed below, we have observed that the mere presence of the HAV sequence in a small cadherin-derived peptide
10 is not the sine qua non for a composition effective to prevent cell-cell adhesion. Indeed, it should be emphasized that neither Blaschuk et al. nor any other publication known to the present inventors suggest that cadherin sequences containing HAV or SHAVS sequences
15 would be effective in opening tight junctions and piercing blood brain barriers formed by E-cadherins in brain microvascular endothelial cells.

SUMMARY OF THE INVENTION

It has now been discovered that molecules
20 homologous to, and immunologically related to, cadherin cell adhesion molecules are present on brain and non-brain microvascular endothelial cells, such that

junctions between such endothelial cells can be reversibly opened so as to permit passage of therapeutic drugs by the use of polypeptide and antibody compositions that compete with such cell
5 adhesion molecules for binding to such cells.

It is therefore an object of this invention to provide the identity of microvascular endothelial cell adhesion molecules.

Another object of this invention is to provide DNA
10 sequences of genes, and plasmids containing same, coding for the expression of all or a cell-binding portion of microvascular endothelial cell adhesion molecules.

Yet another object of this invention is to provide
15 means to identify those sequences of cell adhesion molecules responsible for the tight binding of adjoining endothelial cells.

A further object is to provide therapeutic compositions comprising polypeptides derived from cell
20 adhesion molecules that reversibly disrupt cell-cell adhesion.

Still another object of this invention is to provide therapeutic compositions comprising polyclonal or monoclonal antibodies or fragments thereof directed
25 against endothelial cell adhesion molecules, or against polypeptides representing cell binding regions thereof, that reversibly disrupt endothelial cell-cell adhesion.

Yet another object of this invention is to provide therapeutic formulations comprising therapeutic drugs
30 conjugated with blood-brain barrier-disrupting compositions of this invention, that are capable of entering the central nervous system following disruption of the blood-brain barrier.

These and other objects of this invention will
35 become clear by reference to the following description

of the invention and to the appended claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to chicken N-cadherin.

Figure 2 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to mouse P-cadherin.

Figure 3 illustrates the cDNA sequence for the MDCK cell adhesion molecule homologous to mouse E-cadherin.

Figure 4 illustrates the restriction sites in the bovine endothelial cell N- (4-1 to 4-5) and P-cadherin (4-6 to 4-8) cDNA sequences and in the MDCK E-cadherin (4-9 to 4-14) cDNA sequence.

Figure 5 shows the staining of a mouse brain thin section by an antibody raised against a fusion protein derived from amino acids 9-96 of MDCK E-cadherin containing an HAV region.

Figure 6 is a repeat of the experiment of Fig. 5, except that the antibody was raised against the entire E-cadherin molecule.

Figure 7 illustrates the effects of an 18-mer HAV-containing polypeptide on the resistance of tight junction monolayers of MDCK epithelial cells.

Figure 8 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight junction monolayers MDCK epithelial cells.

Figure 9 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight-junction monolayers of brain microvascular endothelial cells.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that cell adhesion molecules with characteristics of cadherins are present on the surfaces of brain capillary endothelial cells and of microvascular endothelial cells of non-brain origins. The present invention is based on the discovery that a polypeptide composition comprising cell binding domains of endothelial cell adhesion molecules may compete against such molecules for binding to such cells, such that by this means the junctions between such cells could be reversibly opened, thereby permitting penetration by therapeutic agents. The present invention also discloses that polyclonal or monoclonal antibodies (or fragments thereof) raised against endothelial cell adhesion molecules or cell-binding domains thereof may also compete for endothelial cell surface binding sites, and, by this means, reversibly disrupt junctions between endothelial cells, thereby permitting entry into the central nervous system of therapeutic agents.

In order to obtain compositions useful for disrupting tight junctions between microvascular endothelial cells, the cell adhesion molecules responsible for such junctions were identified.

The endothelial cell cadherins disclosed herein exhibit one or more of several characteristics of E-, P- and N- cadherins, including: characteristics of a transmembrane integral protein, with cytoplasmic, hydrophobic plasma membrane, and extracellular regions; intraspecies DNA sequence homologies of greater than about 50% for the entire molecule; immunological cross-reactivity with antibodies raised against non-endothelial cell cadherins; and containing cell-binding domains. "Immunologically related to" means that these cadherin-like molecules cross-react with antibodies

raised against non-endothelial cell cadherins.

E-cadherin-like molecules were localized in brain by immunofluorescence. Cryostat sections of mouse brain were labeled with a rabbit antibody prepared
5 against E-cadherin, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. There is clear labeling of a capillary in brain sections as shown by immunofluorescence microscopy. Endothelial cells in liver and kidney were
10 not stained by this procedure.

cDNAs coding for the expression of bovine microvascular endothelial cell (BMEC) cadherins were cloned and sequenced as described below, and the partial sequence of N-cadherin and P-cadherin are
15 disclosed herein in Figures 1 and 2, respectively. In addition, as MDCK dog kidney epithelial cells are known to employ E-cadherin to form high resistance tight junctions, and as the present invention discloses that brain capillary endothelial cell adhesion molecules
20 include E-type cadherin, the DNA of this cadherin was also cloned; its complete DNA sequence is disclosed herein (Fig. 3).

N-, P- and E-cadherin-type clones described herein were deposited in the American Type Culture Collection
25 on September 26, 1989, and were assigned the following accession numbers:

	<u>Clone Designation</u>	<u>Accession No.</u>
	N-cadherin-type clones	
	pUC19-bNCad 10A	40667
	pUC19-bNCad 39A	40669
5	P-cadherin-type clones	
	pUC18-bPCad 3B-10	40668
	pUC19-bPCad 9B	40670
	E-cadherin-type clones	
	pBluescript MDCKECad 45-30E	40671

10 The cloning of cadherins was accomplished by taking advantage of the fact that the cadherins characterized thus far are transmembrane glycoproteins, the cytoplasmic domains of which are highly conserved, that is, are highly homologous.

15 Two degenerate oligonucleotides flanking the 42-amino acid coding region in the cytoplasmic domain were selected to serve as primers for polymerase chain reaction (PCR) using either BMEC cDNA or MDCK cDNA as templates. The PCR reactions were carried out
20 essentially according to Saiki, R. K. *et al.*, Science, 239:487 (1988), which is incorporated herein by reference.

The cloned PCR products from each cell type were sequenced essentially according to the method of
25 Sanger, F. *et al.*, Proc. Nat'l. Acad. Sci. (USA), 74:5463 (1977), which is incorporated herein by reference.

It was discovered that BMEC cadherins are of two types - one homologous to chicken N-cadherin (neuronal
30 type, see, e.g., Hatta, K., *et al.*, J. Cell Biol., 106:873 (1988)) and the other homologous to mouse P-cadherin (placental type, see e.g., Nose, A., *et al.*, (1987) above). It has also been found that there are two species of cadherins in MDCK cells - one homologous

to mouse E-cadherin (see, e.g., Nagafuchi, A., et al., Nature, 329:341 (1987)) and the other homologous to mouse P-cadherin (Nose, et al. (1987), above).

The PCR products were then used as probes to
5 isolate the BMEC and MDCK cadherin cDNA clones as follows. A cDNA library was constructed essentially according to Gubler et al. (Gubler, U. et al., Gene, 25:263 (1983), which is incorporated herein by reference), using poly (A)⁺RNA isolated from either
10 BMEC or MDCK cells. The cDNA was ligated via EcoRI adaptors into gt10 arms (BMEC) or ZAP^R (from Stratagene, Inc., La Jolla, CA) vector arms (MDCK). cDNA libraries containing 5×10^5 - 1.5×10^6 independent cDNA clones were screened using
15 radiolabeled PCR products (Benton, W.D. et al., Science, 196:180 (1987), which is incorporated herein by reference). Northern blot analysis (Maniatis, T. et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.,
20 1982) may be used to determine whether each cDNA species cloned hybridizes to a single mRNA species, as well as the tissue distributions of each cDNA species.

cDNA clones for each cadherin were sequenced by the method of Sanger et al. (1977) above.

25 The partial restriction maps for each cDNA clone based on their sequences are shown in Fig. 4. Some of these restriction sites were confirmed by restriction enzyme digestions, including Hind III, Pst I, Kpn I, Bgl II for N-cadherin; Pvu II, Sac I and Pst I for
30 P-cadherin; Pst I, Pvu II, BamH I, and Sac I for E-cadherin.

In order to test whether the cloned E-cadherin cDNA contains all the information necessary for cadherin function, full-length E-cadherin cDNA joined
35 to a suitable promoter may be introduced into mouse

L-cells that have very little endogenous cadherin activity (Nagafuchi, et al. (1987), supra). To test for expression of E-cadherin in transfectants derived from the introduced cDNA, transfected L-cells may be
5 tested for Ca^{2+} -dependent aggregating activity. The extent of this aggregating activity should be closely correlated with the amount of E-cadherin expressed (Takeichi, M. (1988), supra). This same technique may be used for testing cDNAs encoding bovine endothelial
10 N- and P-cadherins, according to the method of Hatta, et al. (Hatta, K., et al. (1988), supra).

In order to identify cell binding domains in, for example, MDCK E-type cadherin, L-cells may be first transfected as above with a cDNA of a size sufficient
15 to cause Ca^{2+} -mediated aggregation of transfectants. A series of deletion mutants comprising truncated cDNA species missing different regions of the extracellular domain may be prepared by restriction enzyme digestion and proper end filling or exonuclease digestion to make
20 the deletions in the proper coding frames. These deletion mutants can then be tested for their ability to express in L-cells a protein causing Ca^{2+} -dependent aggregation. By correlating a loss of aggregation with deletion of particular fragments, the regions important
25 for cell binding may be determined. A variety of polypeptides corresponding to binding regions of cadherins, as deduced from the nucleotide sequences of deleted cDNA, may be synthesized chemically using an automated peptide synthesizer such as that of Applied
30 Biosystems, Inc., Foster City, CA, or expressed by recombinant DNA methods. Effective polypeptides may be of varying lengths, depending upon the natures of junctions being disrupted and the cell adhesion molecule present.

Nucleotide, and corresponding amino acid, sequences of cadherins may be analyzed to detect homologous regions. Applying this technique to bovine endothelial cell N- and P-cadherins and to epithelial cell E-cadherin, we have determined that, in the amino acid 80 region of each of these cadherins, there is conserved a triplet HAV (His-Ala-Val) region. We have deduced that this HAV region may be a common cell adhesions recognition (CAR) sequence.

We have chemically synthesized the following polypeptides, each of which containing the HAV sequence:

6-mer(78-83)	NH ₂ -SHAVSS-CONH ₂
11-mer(76-86)	NH ₂ -LYSHAVSSNGN-CONH ₂
17-mer(74-90)	NH ₂ -YILYSHAVSSNGNAVED-CONH ₂
18 mer(69-86)	NH ₂ -EQIAKYILYSHAVSSNGN-CONH ₂
20-mer(71-90)	NH ₂ -IAKYILYSHAVSSNGNAVED-CONH ₂

and have tested each for efficacy in opening brain endothelial cell tight junctions in the BBB model disclosed in copending United States application Serial No. 07/413,274, and also on kidney epithelial cell tight junctions..

Polyclonal antibodies raised in rabbits and monoclonal antibodies derived from hybridomas may be generated against each of the chemically-synthesized polypeptides by standard methods. (Harlow, E., et al., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Goding, J.W., "Monoclonal Antibodies: Principles and Practice", Academic Press, N.Y. 1986). In addition, recombinant antibodies may be prepared. Fragments of antibodies, e.g., Fc, Fab, F(ab)', may be prepared by standard methods.

We have cloned and sequenced fusion proteins derived from amino acids 9-96 of MDCK E-cadherin

containing the HAV region. A polyclonal antibody prepared against this fusion protein stained rat (Fig.55) mouse brain sections as well as did an antibody raised against the entire E-cadherin (Fig. 6).

5 A polyclonal antibody raised against a fusion protein derived from amino acids 9-37 failed to stain brain sections. These results indicate that the key cell-binding domain of E-cadherin lies in the region of amino acids 37-96.

10 The ability of CAM-derived polypeptides containing cell-binding domains, and the corresponding polyclonal and monoclonal antibodies, of the invention to disrupt tight junctions may be tested in in vitro and in vivo models of high resistance tight junctions and in animal
15 models. Monolayers of MDCK dog kidney epithelial cells, that are known to contain high resistance tight junctions (Gumbiner, B., J. Cell Biol., 102:457 (1986)), can be used to test for the ability of the polypeptides and corresponding antibodies of the
20 present invention to disrupt such tight junctions.

Polyclonal antibodies prepared as described above may also be used in conjunction with Western blotting (Old, R.W., et al., Principles of Gene Manipulation, 3d ed., Blackwell, Oxford, 1985, p. 10) and a variety of
25 tissue extracts in order to identify cell adhesion glycoproteins in such extracts.

Another embodiment of the present invention is in drug delivery systems. Conjugates between therapeutic drugs and agents that affect cell adhesion molecule
30 function in brain capillary endothelial cells may be used to deliver therapeutic drugs to the CNS. For example, a polypeptide derived from a cell adhesion molecule that contains within its amino acid sequence a cell-binding domain, or antibodies thereto, may be
35 conjugated in biologically-active form to a therapeutic

modality. Such conjugates may have the dual effect of opening the BBB and delivering the therapeutic agent to the brain side of the BBB. Delivery of therapeutic drugs to the CNS, either alone or conjugated to agents that disrupt cell-cell adhesion, may be accomplished by administering such drugs to a subject either simultaneously with or subsequent to the administration of the agents of this invention that disrupt the tight junctions of the BBB. Examples of therapeutic modalities that may be delivered to the brain by the cell adhesion disruption compositions of this invention include Nerve Growth Factor, anti-Parkinsonian drugs, and brain enzymes known to be missing in sphingolipidoses, e.g., Tay-Sachs disease. Means of chemically conjugating protein or polypeptide carriers to therapeutic agents such that the biological integrity of the therapeutic agent is not compromised and such that the therapeutic agent is readily cleaved from the carrier by enzymes present on or within endothelial cells (e.g., amidases, esterases, disulfide-cleaving enzymes), are well known in the art. It is also apparent that these therapeutic conjugates may be delivered to endothelial cells in encapsulated form (e.g., in liposomes) or as microsuspensions stabilized by pharmacological excipients.

It is known (Jain, R.K., J. Natn'l Cancer Inst., 81:570 (1989)) that many solid tumors develop internal barriers, including high pressure zones and collapsed blood vessels, that make it difficult for blood-borne chemotherapeutic agents to reach the tumor's inner core. The barrier problem is particularly troublesome with therapeutic products drawn from the human immune system, such as monoclonal antibodies conjugated with chemotherapeutic agents, interleukin-2, interferon and activated killer T-lymphocytes, because of their large

size. Thus, in another embodiment of this invention, compositions that disrupt the junctions between endothelial cells, particularly the relatively small peptides that contain one or more cell-binding regions of cell adhesion macromolecules, may be used to enhance drug delivery to tumors with depressed blood flow.

It has been theorized that cancer cells metastasize by secreting soluble cadherins variously to open tight junctions in cells that block their movement and to prevent their being bound to such cells. We consider it likely that antibodies raised against these cadherins, which are derived from extracellular domains of the cadherins disclosed in this invention, may provide a therapeutic modality that inhibits or prevents cancer cell metastases.

In another embodiment, the compositions of this invention may also be used to provide penetration for chemotherapeutic agents of other well-known blood-tissue barriers, such as blood-testis barriers and blood-retina barriers. The latter barrier is known to prevent the efficient transport of, for example, administered antibiotics to the retina from the general circulation. The cell adhesion disrupting compositions of this invention may, thus, be used in conjunction with the administration of antibiotics to treat retinal infections.

The following examples are illustrative of several embodiments of this invention, and should not be construed in any way as limiting the invention as recited in the claims.

EXAMPLE 1

EFFECTS OF HAV-CONTAINING POLYPEPTIDES ON TIGHT JUNCTIONS OF MDCK EPITHELIAL AND BOVINE ENDOTHELIAL CELLS

The BBB model of compending U.S. Serial No. 07/413,332 was used to examine the effects of polypeptides containing the HAV region on the tight junctions of monolayers of MDCK epithelial cells and
5 bovine capillary endothelial cells as determined by resistance measurements across the monolayers.

The polypeptide was added to the cells either from the apical side (top) or basolateral side (bottom), as shown in the following sketch.

10

APICALEPITHELIAL CELLS
Gut SideENDOTHELIAL CELLS
Blood Side

Blood Side

Brain Side

BASOLATERAL

15 Figure 7 illustrates the effects of various concentrations of the aforementioned 18-mer polypeptide on resistance of MDCK epithelial cells. At the lowest concentration tested, 0.5 mg/ml, resistance was markedly decreased. The polypeptide was more effective
20 when added from the basolateral side, but at high concentrations was quite effective even when added from the apical side. These data indicate that the 18-mer is effective in making tight junctions permeable. The 20-mer was similarly effective, and a 17-mer less
25 effective.

Figure 8 illustrates the effects of the aforementioned 11-mer and 18-mer on MDCK cell resistance when added from either the apical or
basolateral side of the monolayers. The concentration
30 of polypeptide was about 1 mg/ml. The 11-mer (as well

as the 6-mer data not shown) was virtually without effect. With the 18-mer, resistance was almost totally abolished by about 6 hours, indicating disruption of tight junctions. That the effect of the 18-mer is reversible is indicated by the "wash-out" experiment. When the 18-mer was washed out of the MDCK cells at 6 hours, resistance recovered to a substantial extent over the next 21 hours. This recovery was particularly pronounced when the 18-mer had originally been added from the basolateral side of the monolayers. The 20-mer produced results similar to those of the 18-mer, and the 17-mer was effective, but somewhat less so.

Figure 9 illustrates the effect of 1 mg/ml of the 11-mer and 18-mer on high resistance monolayer cultures of brain endothelial cells (see copending United States Serial No. 07/413,332 for method of preparation). As with MDCK cells, the 11-mer (and the 6-mer) failed to reduce resistance values over a 48-hour period of observation. In contrast, the 18-mer (as well as the 20-mer) decreased resistance values markedly when added from either the basolateral or apical side, but the effect of the polypeptide was more rapid and more pronounced when it was added from the basolateral side; the 17-mer was less effective.

The conclusion of these experiments is that a particular set of peptides (but not all peptides) centered around the HAV region of E-cadherin are effective in opening tight junctions of brain endothelial cell blood-brain barriers, and also of epithelial cells that form such junctions ("gut barrier"). Both the length and composition of the amino acid region flanking the HAV triplet thus appear to play a role in the efficacy of such compositions.

While the aforementioned embodiments represent the preferred embodiments of the invention, those skilled

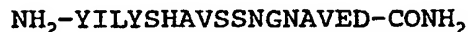
in the art may, without undue experimentation, devise other executions of the compositions and methods of use of this invention without departing from the concept and spirit inherent therein.

What is claimed is:

1. A composition for opening tight junctions between microvascular endothelial cells of a subject, whereby means are provided for a drug to cross the permeability barrier imposed by such junctions,
5 comprising an agent capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted.
2. A composition of claim 1, wherein said cell adhesion molecule exhibits at least about 50% sequence homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.
3. A composition of claim 1, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.
4. A composition of claim 1, wherein the microvascular endothelial cells are brain capillary endothelial cells.
5. A composition of claim 2, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
6. A composition of claim 3, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
7. A composition of claim 5, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.
8. A composition of claim 7, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

9. A composition of claim 8, wherein said cell-binding domain contains an HAV amino acid sequence.

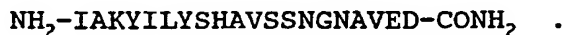
10. A composition of claim 9, wherein said amino acid sequence is



11. A composition of claim 9, wherein said amino acid sequence is



12. A composition of claim 9, wherein said amino acid sequence is



13. A composition of claim 9, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

14. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

15. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

16. A composition of claim 15, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

17. A composition of claim 16, wherein said cell-binding domain contains an HAV amino acid sequence.

18. A composition of claim 17, wherein said amino acid sequence is



19. A composition of claim 17, wherein said amino acid sequence is



20. A composition of claim 17, wherein said amino acid sequence is



21. A composition of claim 17, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

22. A composition of claim 5 or 6 in a pharmaceutically-acceptable vehicle.

23. A method for opening tight junctions between microvascular endothelial cells of a subject, comprising the step of administering to the subject an agent, in an effective amount and in a
5 pharmaceutically-acceptable vehicle, capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted and
10 whereby means are provided for a drug to cross permeability barriers imposed by such tight junctions.

24. A method of claim 23, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

26

25. A method of claim 23, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

26. A method of claim 23, wherein the microvascular endothelial cells are brain capillary endothelial cells.

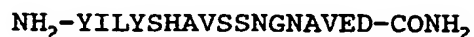
27. A method of anyone of claims 23-25, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

28. A method of claim 27, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

29. A method of claim 28, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

30. A method of claim 29, wherein said cell-binding domain contains an HAV amino acid sequence.

31. A method of claim 30 wherein said amino acid sequence is



32. A method of claim 30, wherein said amino acid sequence is



33. A method of claim 30, wherein said amino acid sequence is



34. A method of claim 30, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

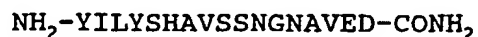
35. A method of claim 27, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

36. A method of claim 28, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said fragment of said cell adhesion molecule.

37. A method of claim 36, wherein said cell adhesion fragment includes within its amino acid sequence a cell-binding domain.

38. A method of claim 37 wherein said cell-binding domain contains an HAV amino acid sequence.

39. A method of claim 38, wherein said amino acid sequence is



40. A method of claim 38, wherein said amino acid sequence is



41. A method of claim 38, wherein said amino acid sequence is



42. A method of claim 38, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

43. A drug delivery composition comprising a conjugate between a therapeutic drug and an agent capable of reacting with at least one type of a cell-bound cell adhesion molecule that would otherwise
5 mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is

disrupted by said agent, whereby means are provided for said drug to cross permeability barriers imposed by such tight junctions, in a pharmaceutically-acceptable vehicle.

44. A drug delivery composition of claim 43, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

45. A drug delivery composition of claim 43, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

46. A drug delivery composition of claim 43, wherein the microvascular endothelial cells are brain capillary endothelial cells.

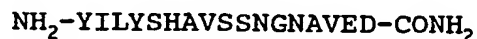
47. A drug delivery composition of any one of claims 43-45, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

48. A drug delivery composition of claim 47, wherein said agent comprises a fragment of said cell adhesion molecule.

49. A drug delivery composition of claim 48, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

50. A drug delivery composition of claim 49, wherein said cell-binding domain contains an HAV amino acid sequence.

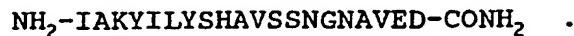
51. A drug delivery composition of claim 50, wherein said amino acid sequence is



52. A drug delivery composition of claim 50, wherein said amino acid sequence is



53. A drug delivery composition of claim 50, wherein said amino acid sequence is



54. A drug delivery composition of claim 50, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

55. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

56. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

57. A drug delivery composition of claim 56, wherein said cell adhesion molecule fragment contains within its amino acid sequence a cell-binding domain.

58. A drug delivery composition of claim 56, wherein said cell-binding domain encompasses an HAV amino acid sequence.

59. A drug delivery composition of claim 58, wherein said amino acid sequence is



60. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH₂-EQIAKYILYSHAVSSNGN-COHN₂ .

61. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH₂-IAKYILYSHAVSSNGNAVED-CONH₂ .

62. A drug delivery composition of claim 58, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

63. A drug delivery composition of claim 43, wherein said conjugate comprises a physiologically-cleavable covalent bond.

64. A drug delivery composition of claim 43, wherein said conjugate is encapsulated within a physiologically-compatible particle.

65. A drug delivery composition of claim 64, wherein said particle comprises a liposome.

FIG. 1a.

Partial cDNA sequence for the bovine endothelial N-cadherin

GAATTGGAAC	CCCTTCGTTT	CATTATGCAA	GACTGGATT	CCTGAAGATG	TGTACAGTGC	60
AGTCTTGTC	CGGGATGTGC	TGGAAGGACA	GCCCCTTCTC	AATGTGAAGT	TTAGCAACTG	120
CAATGGGAAA	AGAAAAGTAC	AGTATGAGAG	CAGCGAGCCA	GCAGATTTTA	AGGTGGATGA	180
AGATGGCATG	GTGTATGCCG	TGAGAAGCTT	CCCCCTCTCA	TCTGAACACT	CGAAGTTCCT	240
GATATACGCT	CAAGACAAAG	AGACTCAGGA	AAAGTGGCAA	GTAGCAGTAA	AACTGAGCCT	300
CAAAACCAGCC	CTACCTGAGG	ATTCAGTGAA	GGAATCACGA	GAAATAGAAG	AAATAGTGTT	360
TCCAAGACAA	GTGACTAAGC	ACAAATGGCTA	CCTGCAGAGG	CAGAAGAGAG	ACTGGGTTAT	420
CCCTCCCATC	AACTTGCCAG	AAAACCTCCAG	AGGGCCCTTTT	CCTCAAGAGC	TCGTCAGGAT	480
CAGATCCGAT	AGAGATAAAA	ACCTTTCTCT	GCGGTACAGC	GTAACCTGGC	CAGGAGCTGA	540
CCAGCCCTCCA	ACTGGTATCT	TCATTATCAA	CCCCATCTCA	GGTCAGCTGT	CAGTAACCAA	600
GCCTCTGGAT	CGTGAGCTGA	TAGCCCCGTT	TCATTTGAGG	GCACATGCAG	TGGATATTAA	660
TGGAAACCAA	GTGGAGAACC	CCATCGACAT	TGTCATCAAC	GTTATTGACA	TGAATGATAA	720
CAGACCTGAG	TTCTTACACC	AGGTTTGGAA	TGGGACAGTT	CCTGAGGGAT	CAAAGCCGGG	780
AACATATGTG	ATGACGGTCA	CTGCGATTGA	TGCTGACGAT	CCAAATGCCC	TCAATGGGAT	840

1/42

FIG. 1b.

GTGAGGTAC AGAATCCTGT CCCAGGCGCC AAGCACCCCT TCGCCCAACA TGTTTACAAT	900
CAACAATGAG ACTGGGACA TTATCACGGT GGCAGCTGGA CTTGACAGAG AAAAAGTACA	960
ACAGTATACG TTAATAATTC AAGCTACAGA CATGGAAGGC AATCCACAT ATGGCCTTTC	1020
CAACACAGCC ACGGCTGTCA TCACGGTGAC AGATGTCAAC GACAATCCTC CGGAGTTTAC	1080
TGCCATGACG TTCTATGGTG AAGTCCCCTGA AAACAGGGTA GATGTCATCG TCGCTAATCT	1140
AACAGTGACA GATAAGGATC AGCCCCACAC ACCGGCCTGG AACGCCATCT ACAGAAATCAG	1200
CGGTGGAGAC CCCGCCGGCC GCTTTGCCAT TCAAACTGAC CCCAACAGCA ACGACGGTTT	1260
AGTCACCCGTA GTAAAACCAA TCGACTTTGA AACAAATAGG ATGTATGTCC TTACTGTGCG	1320
TGCAGAAAAT CAAGTGCCAT TAGCCAAGGG TATTCAGCAT CCACCTCAGT CAACTGCGAC	1380
TGTGTCTGTC ACAGTTATCG ATGTGAATGA AAATCCTTAT TTTGCCCCAA ATCCAAAGAT	1440
CATTGCGCAA GAAGAAGGCC TTCACGCCGG TACCGTGTTA ACAACGTTTA CTGCTCAGGA	1500
CCCAGATCGA TATATGCAGC AAAATATCAG ATACACCAA TTATCCGATC CTGCAAACTG	1560
GCTAAAAATA GACTCTGTGA ATGGCAGAT AACTACCAT GCTGTTTGG ACAGAGAATC	1620
ACCGAATGTG AAAGCCAATA TATACAATGC TACTTTCCTT GCTTCTGACA ATGGAATCCC	1680
TCCTATGAGT GGAACGGGAA CACTGCAGAT CTATTTACTT GATATTAATG ACAATGCCCC	1740

2/42

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FIG. 1c.

TCAAGTGTTA	CCTCAAGAGG	CAGAGATTGG	TGAAACTCCG	GACCCCAATT	CAATTAACAT	1800
CACAGCACTT	GATTATGACA	TTGATCCAAA	TGCTGGACCA	TTTGCTTTTG	ATCTTCCTTT	1860
GTCTCCAGTG	ACTATTAAGA	GAAATGGAC	CATCACTCGG	CTTAATGGTG	ATTTTGCTCA	1920
GCTTAACTTA	AAGATAAAAT	TTCTTGAGGC	CGGGATCTAC	GAAGTTCCAA	TCATAATCAC	1980
AGATTGCGGT	AATCCTCCCA	AATCGAATAT	CTCCATCCTT	CGGGTGAAGG	TTTGCCAGTG	2040
TGATTCCAAC	GGGACTGCA	CAGATGTGGA	TCGAATTGTG	GGAGCAGGC	TGGGCACCGG	2100
CGCCATCATC	GCCATCCTGC	TTTGCAATCAT	CATCCTGCTC	ATTCTCGTTC	TGATGTTCTG	2160
GGTATGGATG	AAACGCCGGG	ATAAAGAACG	CCAGGCCAAA	CAACTTTTAA	TTGATCCAGA	2220
AGATGATGTA	AGAGATAATA	TTTTAAATA	TGATGAAGAA	GGTGGAGGAG	AAGAAGACCA	2280
GGACTACGAT	TTGAGCCAGC	TCCAGCAGCC	TGATACGGTA	GAGCCAGATG	CCATCAAGCC	2340
AGTTGGAATC	CGACGGTTGG	ATGAGAGGCC	CATCCATGCG	GAGCCCCAGT	ACCCGGTTCG	2400
ATCTGCAGCC	CCACACCCAG	GGGACATCGG	GGACTTCATT	AATGAGGGCC	TTAAAGCTGC	2460
TGACAACGAT	CCCACCGCTC	CGCCCTACGA	CTCCCTCTTA	GTCTTTGACT	ATGAAGGCAG	2520
TGGCTCCACG	GCCGGGTCCT	TGAGCTCCCT	TAATTCCTCC	AGTAGTGGAG	GTGAGCAGGA	2580
CTATGACTAT	CTGAACGACT	GGGGGCCCCG	CTTCAAGAAA	CTCGCTGACA	TGTACGGTGG	2640

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AGGTGATGAC TGAACCTCAG GGTGAACCTTG GTTTTITGGAC AAGTACAAAC AATTGCAACT 2700
GATATTCCCA AAAAGCATTC AGAAGCTAGG CTTTAACTTT GTAGTCTACT AGCACAGTGC 2760
TTGCTGGAGG CTTTGGCAGA GGCTGCAAAC CAATTITGGC TCAGAGGGAA TATCGGTGAT 2820
CCAATACTGT TTGGAAAACA CTGAGCTCAG TTACACTTGA ATTTTACAGT ACAGAAGCAC 2880
TGGGATTTTA TGTGCCCTTTT TGTACCTTTT TCAGATTGGA ATTAGTTTAA TGTTTAAGGC 2940
TTTAAATGGTA CTGATTTC TG AATGATAAG TAAAAGACAA AATATTTTGT GGTGGGAGCA 3000
GTAAGTTAAA CCATGATATG CTTGACACAG CTTTGTGTAC ATCGCATTTG CTTTATTAA 3060
AAATATGGAA TTAAACAGAC AAACCAACCA CTCATGAGC AATTTTATTA CCTTGGGGGC 3120
TGAGACCATG AGATTGGAAA ATGTACATTA TTTCTAGTTT TAGACTTTAG TTTCTTGTTT 3180
TGTTTTTTTT TTCCACTAAA ATCTTAAAC TTACGCAGCT GGTGCAAAT AAAGGGAGTT 3240
TTCATATCAC CAATTGTAG CAAAATTGAA TTTTTTCATA AACTAGAATG TTAGACACAT 3300
TTTGGTCTTA ATCCATGTAC ACTTTTTTAT TTA CTGTATT TTTTCCACTT CACTGTAAA 3360
ATGGTATGTG TACATAATGT TTTATTGGCA TAGTCTATGG AGAAGTGCAG AAACCTCAGA 3420
ACATGTGTAT GTATTATTG GACTATGGAT TCAGGTTTTT TGCATGTTTA TATCTTTCGT 3480
TATGGATAAA GTATTACAA AACAAAGTGA CATTGATTC AATTGTTGAG CTGTAGTTAG 3540
AATACTCAAT TTTTAATTTT TTAATTTTTT TTATTTTTTA TTTTCTCTTT TTGTTTGGG 3600

FIG. 1d.

4/42

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AGGGAGAAAA GTTCTTAGCA CAAATGTTTT ACATAATTG TACCAAAAAA AACAAAAAA 3660
AAAGGAAAGA CAAGAAATGA AAGGGGTGAC CTGACACTGG TGGTACTACT GCAGTGTGTG 3720
TTTTTAAAAA AAAATGAAAA AAAAAAAGCT TTAAACTGG AGAGACTTCT GACAACAGCT 3780
TTGCCCTCTGT ATTGTGTACC AGAATATAAA TGATACACCT CTGACCCACG CGTTCTGAAT 3840
AAAAATGCTAA TTTTGGAAAA AAAAAAAA AAAA 3875

FIG. 1e.

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FIG. 2a.

partial cDNA sequence for the bovine endothelial P-cadherin

GAATTGGAAC CCCTTCGCTG AGAACACAGT GAGCCACGAG GTGCAGAGGC TGACAGTGAC 60
TGATCTGGAC GCCCCTAACT CACCAGCATG GCGTGCCACC TACCGCATCG TGGGAGGTGA 120
CAACGGGGAC CATTTTACCA TCACTACTGA CCCCAGAGAGC AACCAGGGTA TCCTGACCAC 180
CCAGAAGGGC TTGGATTTTG AGGCCAAAAC CCAGCACACC CTGTACGTCG AAGTGATCAA 240
CGAGGTCCC TTTGTGGTGA AACTCCCGAC CTCCACAGCC ACCGTAGTGG TCCTCGTGGA 300
GGATGTGAAT GAGCCACCEG TGTGTGTCCC CCCGTCCAAA GTCATCGAAA TCCAGGAGGG 360
CATCTCCACT GGGGAGCCTA TTTGTGCCTA CACTGCACGG GACCCAGACA AGGGAGTCA 420

FIG. 2b.

6/42

GAAGATCAGT	TACCACATCC	TGAGAGACCC	AGCAGGGTGG	CTAGCGATGG	ACCCAGACAG	480
TGGACAAGTC	ACTGCCGCAG	GGGTCTTGGA	CCGTGAGGAT	GAGCAGTTTG	TGAGAAAACAA	540
CATCTACGAA	GTCATGGTCT	TGGCCACAGA	TGATGGGAGC	CCTCCCACCA	CTGGCACAGG	600
GACCCCTCTG	CTAACACTGA	TGGACATCAA	TGACCACGGT	CCGGTCCCCG	AGCCCCGTCA	660
GATCACCATC	TGCAACCAA	GCCCTGTGCC	CCAGGTGCTA	AACATCACAG	ACAAGGACTT	720
GTCCCCCCAC	ACTGCCCCCTT	TCCAGGCCCA	ACTCACACAT	GACTCGGACG	TCTATTGGAC	780
AGCAGAAGTC	AACGAGAAAG	GAGACGCAGT	AGCCTTGTC	CTGAAGAAGT	TCCTAAAGCA	840
AGGCGAATAC	GATGTGCACC	TTTCCCTGTC	CGACCACGGC	AACAAGGAAC	AGCTGACAGT	900
GATCAGAGCC	ACCGTGTGTG	ACTGCCACCG	CAACATGGTG	ACCTGCCGGG	ACCCCTGGAC	960
GTGGGGTTTC	CTCCTCCCCA	TCCTGGGTGC	TGCCCTGGCT	CTGCTGCTCC	TTCTGCTGGT	1020
GCTCCTATTC	TTGGTGAGAA	AGAAACGGAA	GATCAAGGAA	CCCCTTCTCC	TCCCAGAAGA	1080
TGATACCCCGT	GACAACGTCT	TCTACTACGG	CGAAGAGGGG	GGTGGCGAGG	AGGACCAGGA	1140
CTATGACATC	ACCCAGCTCC	ACCGGGGTCT	GGAGGCCCGG	CCTGAGGTGG	TTCTCCGCAA	1200
CGATGTGGCA	CCATCCTTCA	TCCCCACACC	CATGTACCGT	CCTCGGCCAG	CCAACCCAGA	1260
TGAAATCGGC	AACCTCATCA	TTGAGAACCT	GAAGGCAGCC	AACACAGACC	CCACGGGCCCC	1320

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CCCCTACGAC TCCCTGTTGG TGTTCGACTA TGAGGGCAGT GGCTCCGATG CCGCCTCTCT 1380
GAGCTCGCTC ACCTCCTCAA CCTCTGACCA GGACCAAGAC TACAACTATC TGAATGAGTG 1440
GGCAGCCGC TTCAAGAAGC TGGCGGACAT GTACGGCGGG GGCCAGGACG ACTAGGACTC 1500
CCTAAACGCC GGGCTGCAGC AGCGTCTCCA AGGGTCACT ATCCCCACGT TGGCCAAGGA 1560
CTTTCAGCT TGTGAGAAAT TGGCCTTAGC AACTTGGAGG GAAGAGGCCT CGAAACTGAC 1620
CTCAAAGGG CAGGTCTCTA TGCCTTTCAG AACGGAGGAA CGTGGGCAGT TTGATTTCAA 1680
CAGTGAGCAC CTCTTAGCCT AAGCCAGGGC TGCTCAATT CTGGGAGTCT CCTCGCTACC 1740
ATAAAATGCT CAGCGCTGGG TCCTGGGTTT TGA CTGACTC TGA CTTTCCC ATGATGGCTT 1800
TTGCTCTGGA ATGACCCCTT CTCCTTAGTA ACAGGCCTCT TACCACAATC TTCGTTTTTT 1860
TTTTTTTAAAT GCTGTTTTCA AAAAGTGAGA GGCAGGTCCT CAACCACCCC CTGGAGCGCT 1920
CCAGAAAGCCC AGGCGTGCCC TCATGCATTT CTCTGTGGTC TCTTGGCCCC CAGACCTCCT 1980
GTTTGATTGG ATAACTGCAT TTTTATACTG AGCACGTCTA AGTGGTCCTT TATTTTAT 2040
TTTCCCTATC GAGTGCTGTA GATGAAGAGT GATGACAATC CTGTAAATGT ACTAGAACTT 2100
TTTTTATTAA GGAACTTTTT CCCAAAAAAA AAAAAAAA AAAAAC 2156

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FIG. 2c.

FIG. 3a.

cdna sequence for MDCK E-cadherin

CGGGCACCTG	TGATTGCGG	AAGTCCTGCC	GCCTCGCGCC	GCCTCGCGCC	CGGCTCTCGA	60
CCCCCGCCCG	CCATGGGCCC	TCGGTACGGC	GGCGCCCCCG	CGCTCCTGCT	CCCGCTGCTG	120
CTGCTGCTGC	AGGTCTCATC	GGGGCTCTGC	CAAGAGCCCG	AGCCCTGCCG	CCCTGGCTTT	180
GGCGCTGACA	GCTACACGTT	CACCGTGCCC	CGCGACACT	TGGAGAGAGG	CCGTGTCCTG	240
GGCAGGGTGA	GTTTTGAAGG	ATGCACCGGT	CTACCTAGGA	CAGCCTATGT	TTCTGATGAC	300
ACCCGATTCA	AAGTGGGCAC	AGATGGTGTG	ATTACAGTCA	AGCGGCTCT	ACAACTTCAT	360
AAACCAGAGA	TAAGTTTCT	TGTCCATGCC	TGGGACTCCA	GCCGCAGGAA	GCTCTCCACC	420
AGAGTTAGGC	TGAAGGCAGC	GACGCACCAC	CACCACCACC	ATCATGATGC	TCCCTCTAAA	480
ACCCAGACAG	AGGTGCTCAC	ATTGCCCAGT	TCCCAGCATG	GACTCAGAAG	ACAGAAGAGA	540
GACTGGGTTA	TCCCTCCTAT	CAGCTGCCCG	GAAAACGAGA	AAGCCCCATT	TCCTAAAAC	600
CTGGTTCAGA	TCAAGTCTAA	CAGGGACAAA	GAAATCAAGG	TTTTTCTACAG	CATCACTGGC	660
CAAGGAGCTG	ACGCACCTCC	TGTTGGTGTG	TTTATTATTG	AAAGAGAAAC	AGGATGGCTG	720
AAGGTGACTG	AGCCTCTGGA	TAGAGAAACAA	ATTGCTAAGT	ACATTCTCTA	CTCTCATGCC	780
GTATCTTCTA	ATGGGAATGC	GGTTGAAGAC	CCAATGGAGA	TCGTGATCAC	GGTGACAGAT	840

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FIG. 3b.

CAGAAATGACA	ACAAGCCCCGA	GTTCACCCAG	GCAGTCTTCC	AAGGATCTGT	CACGGAAGGT	900
GCCCTTCCAG	GCACCTCTGT	GATGCAGGTG	ACAGCCACAG	ATGCGGATGA	TGATGTGAAT	960
ACCTACAACG	CTGCCATCGC	TTACAGCATC	CTCACACAAG	ACCCCTCCT	GCCTAGCAGC	1020
ATGATGTTCA	CTATCAACAA	GGACACAGGA	GTCAATCAGG	TGCTCACCAC	TGGGCTGGAC	1080
CGAGAGGGTG	TCCCCATGTA	CACCTTGGTG	GTTCAGGCTG	CTGACCTGCA	AGCGGAAGGC	1140
TTAACTACAA	CTGCAACAGC	TGTGATCACA	GTCACTGACA	TCAATGATAA	CCCCCCCATC	1200
TTCAACCCAA	CCACGTACCA	GGGACGGGTG	CCTGAGAACA	AGGCTAACGT	CGAAATCGCT	1260
GTA CTCAAAG	TGACGGATGC	TGATGTCCCC	GATACCCCGG	CCTGGAGGGC	TGTGTACACC	1320
ATATTGAACA	ATAACAATGA	TCAATTGTGT	GTCAACCCAG	ACCCAGTAAC	TAAACGACGC	1380
ATTTTGAAAA	CAACTAAGGG	CTTGGATTTT	GAGGACAAGC	AGCAGTATGT	CTTGTACGTG	1440
ACTGTGGTGA	ACGTGACCCC	GTTTGAGGTC	ATCCTCTCCA	CCTCCACAGC	CACTGTCACT	1500
GTGGACGTGG	AAGATGTGAA	TGAAGCCCCC	ATCTTCATCC	CTTGCCCCAA	GGTAGTGTCA	1560
ATCCCCTGAAG	ACTTTGGTGT	GGGCCAGGAA	ATCACATCCT	ACACCGCCGA	GGATCCAGAT	1620
ACATATATGG	AACAGAGGAT	AACGTATCGG	ATTTGAGGG	ATGCTGCCGG	TTGGCTGGAG	1680
GTTAATCCAG	AATCTGGTGC	CATTTTCACT	CGGGCTGAGC	TGGACAGAGA	GGATTTTGAG	1740

9/42

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FIG. 3c.

CACGTGAAGA ATAGCACGTA TGAAGCCCTC ATTATAGCCA TTGACTTCGG TTCTCCAGTT 1800
GCTACTGGAA CGGGAAC TCT TCTACTGGTC CTCTCTGATG TGAATGACAA TGGCCCCATT 1860
CCAGAACCTC GAAATATGGA CTTCTGCCAG AAAAACCCAC AGCCTCATGT CATCAACATC 1920
ATTGATCCAG ATCTTCCCC CAACACATCT CCCTTCACAG CAGAACTAAC ACACGGCGCA 1980
AGTGTCAACT GGACCATCGA GTACAATGAC CCAGCTCGTG AATCTCTAAT TTTGAAGCCA 2040
AAGAAAACTT TAGAGTTGGG TGACTACAAA ATAAATCTCA AGCTCACAGA TAACCAGAAC 2100
AAGGACCAGG TGACCACCCT ATATGTGTTT GTGTGCGACT GCGAAGGTGT CGTCAACAGC 2160
TGCAAGAGGA CGGCGCCTTA CGCCGAAGCA GGCTTGCAGG TTCTTGCCAT CTTGGGCATT 2220
CTCGGAGGAA TCCTCGCTCT ACTAATCCTG ATTCTGCTGC TTCTGCTATT TGTTCGGAGG 2280
AGAAGGGTGG TCAAAGAGCC CTTACTTCCC CCAGAAGATG ACACCCGGGA CAATGTTTAT 2340
TACTATGATG AAGAAGGAGG TGGAGAGGAG GATCAGGACT TTGACTTGAG CCAGTTGCAC 2400
AGGGGCCCTGG ATGCTCGGCC TGAAGTGACT CGCAATGATG TGGCCCCAAC CCTCCTGAGT 2460
GTGCCCCCAGT ATCGGCCCCG CCTGCGCAAT CCTGATGAAA TTGGAACCTT TATTGATGAA 2520
AACCTGAAGG CAGCGGACAC TGACCCCTACT GCTCCTCCTT ATGACTCTCT GCTCGTGTTT 2580
GACTATGAAG GAAGCGGTTT TGAAGCTGCT AGTCTGAGCT CCTTGAACTC CTCAGAGTCA 2640
GACCAAGACC AGGACTATGA CTACCTGAAT GAATGGGGCA ATCGCTTCAA GAAGCTGGCG 2700

10/42

FIG. 3d.

GACATGTATG GAGGTGGCGA GGACGACTAG GGGACTTGAG ACAAATGAAG ATGAGTCCTT 2760
ATACCATGTG GTAGAAAATG CGGAGGTGAC TGTTTTCAGC TCCCTTCATC TGAGAGGAAT 2820
TTCTGGAGAA GAGAAAATGC ACAGTGATAT ATAGTTAGGA TAGTTAGGAT TTCTACTTTA 2880
TAGATCTAAT CTGTGTGTTT GTTAGAACGA TTTTGACCTA TTCTTTGAAG CTTTTTTTTC 2940
TTTCTTTTCAT CATTCCTTAA ATGGTGATGC TGTCCAAAAG ACCCCCCACA TGTTTATATT 3000
TCAAAAGAAT AGCTAAAGCC TCCAGAAGGT TCTGCTAGCA ATTTCCGAGAT TGCCTTATTG 3060
ACTTGCTCTCA TTTTTTTTAAA GGAAGGTAGG GCTAAACTAC CCTATTGTGT TTGTGTGTGT 3120
GTGTGTGTAT GTGTAATTAT TTTTAAATTG TGTCTCTTTT TCTCCTATCA CTGCACTGGT 3180
GTCCCGTGTT CTAATAACCA CTCCTAACTC CTCTGAACT TACATTGCCT CAGACAGGAG 3240
TTCTCTGCTG CAGAAATTAT TGGGCCCTTT CAGGATAAGA GACTTGTCT TAGTTTGATG 3300
GTAGTGTGAC TGGGTATTAT GGACTCGTAA GGACTTTAGT GGTCTCCTT TTTTATTTC 3360
TAAGTACATA AATTGAAATT CATATCCATC CACTGACTTG TTCTGCATTA AGTGTGTTTG 3420
TCATGTGGAC GTCATTATTG GGCTACTTTG GTTCTGAACA AGGAGCATTG ACCAGAAAAG 3480
GTGGTGAATT TTCAGGTGCC ACTCAACTTC TAATGTTTAC TTATCACTCA AACAGAAAG 3540
TGATCTATTC TGACGTTTAG CGTAGTGCCT GCAGTGCTGC AGCCAAAGAT TGAAGGCGGA 3600

11/42

SUBSTITUTE SHEET

12/42

TTGTCAAAGC CAAGGGCAAC ATGAAAAATG GACTTGGAGG TGGCAGGCGG GATGGGTCAT 3660
TGAGCCTGGC GTTTTAGCAA ACTGATGCTG AGGATAACTG AGGTGGCTCT ACCTCTAGTC 3720
CTGAAAATTC TGAAGAATGG AAGAATCCCG ACAAGTGTTT CCTATCGCGA TCCTTAGGTC 3780
ACAGTTTGTA CCTGAGGCCA AGAATCCCCA GGTCCTGCTT TTTGTTAATG TCTACCGAAA 3840
ATGCAGCCTG ATCTGGACTC AGGTGCCCCA ATTCTAAGTG TGCATAGAAA ACTGACAATA 3900
TTAGGAAATT CTTTTTCCCC CCTTAGGAGC AGGAAGAAAA TATGACCCTA AAGGGTTTGT 3960
GCAAAGGAA GGTGGGGAGA GCTTTGACTT GGATTTTTTT TAAATTGAAA TGTGAACCTC 4020
AAGGAACTTT TGACAACCAT GGGAAATAAT TTTATCTTAA ATTGCTTTAC TGTCTGTCAG 4080
CTGTTTTTCA AAGAAAAAAA AAATCATCCC TGCAATCACT TCTTGGAATT GTCTTGATTT 4140
TTCAGCAATT TAAACTCTAA TTTAGTCCTG TATAGAGAAAT GTTAATGTAG TTTTGAGTGT 4200
ATATGTGTGT GGGTACGGAT AATTTGTAT TTTCTTTAGG TCTGGAAAAG GAAAACAATT 4260
TAAGCTGCGA AAATTCTTAA ATATTCATTT TTATAAATTT TATTAAGAA TTTTGTAAAA 4320
AAAAAAAA AAA 4333

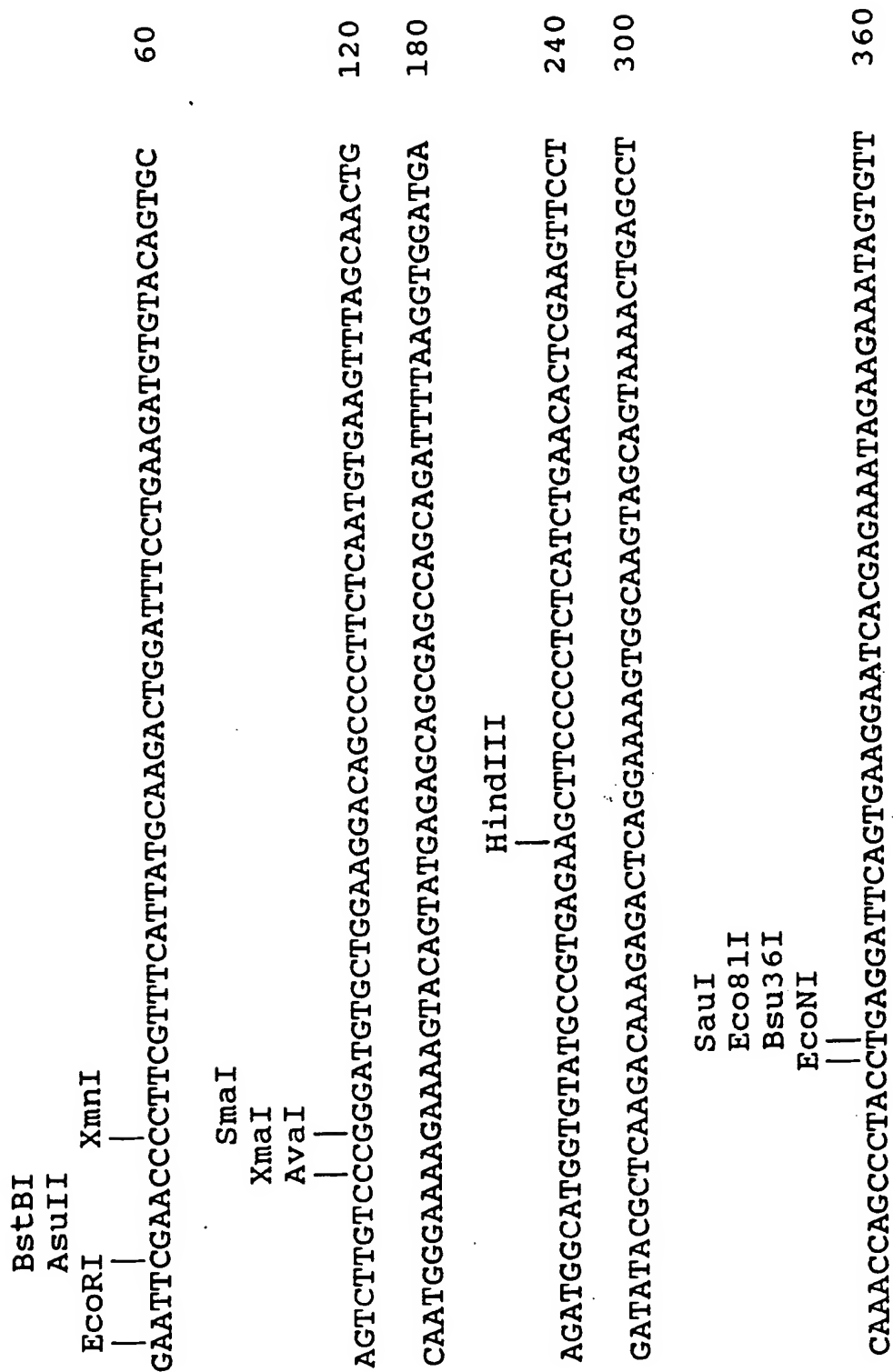
FIG. 3e.

SUBSTITUTE SHEET

13/42

FIG. 4a.

N-cadherin restriction map



14/42

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FIG. 4c.

15/42

GCCTCTGGATCGTGAGCTGATAGCCCGGTTTCATTTGAGGGCACATGCAGTGGATATTAA 660
 Tth1111I
 TGGAACCAAGTGGAGAACCCCATCGACATTGTGCATCAACGTTATTGACATGAATGATAA 720
 SauI
 Eco81I
 Bsu36I
 AlwNI
 CAGACCTGAGTCTTACACCAGGTTTGGAATGGGACAGTTCCTGAGGGATCAAAGCCGGG 780
 NdeI
 AACATATGTGATGACGGTCACTGCGATTGATGCTGACGATCCAAATGCCCTCAATGGGAT 840
 HaeII
 BbeI
 NarI
 BamI
 EcoNI AhaII
 NspHI
 AflIII
 GTTGAGGTACAGAAATCCTGTCTCCAGGCGCCAAGCACCCCTTCGCCCAACATGTTTACAAT 900
 PvuII
 CAACAATGAGACTGGGGACATTATCACGGTGGCAGCTGGACTTGACAGAGAAAAGTACA 960

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FIG. 4d.

AccI | NdeI | 1020
 ACAGTATACGTTAATAATTCAAGCTACAGACATGGAAGCAATCCCACATATGGCCTTTC

HincII | BspMII | AccIII | 1080
 CAACACAGCCACGGCTGTCTATCAGCGTGACAGATGTCAACGACAATCCTCCGGAGTTTAC

TGCCATGACGTTCTATGGTGAAGTCCCTGAAAAACAGGGTAGATGTCTCGTCGCTAATCT 1140

Cfr10I | 1200
 AACAGTGACAGATAAGGATCAGCCCCACACACCGGCTGGAAACGCCATCTACAGAATCAG

NaeI | Eco52I | EagI | Cfr10I | 1260
 CGGTGGAGACCCCGCGCGCTTTGCCATTCAAACTGACCCCAACAGCAACGCGTTT

PstI | StyI | HincII | 1320
 AGTCACCGTAGTAAACCAATCGACTTTGAAAACAAATAGGATGTATGTCTTACTGTCCG

TGCAGAAAATCAAGTGCCATTAGCCCAAGGGTATTTCAGCATCCACCTCAGTCAACTGCCGAC 1380

16/42

FIG. 4e.

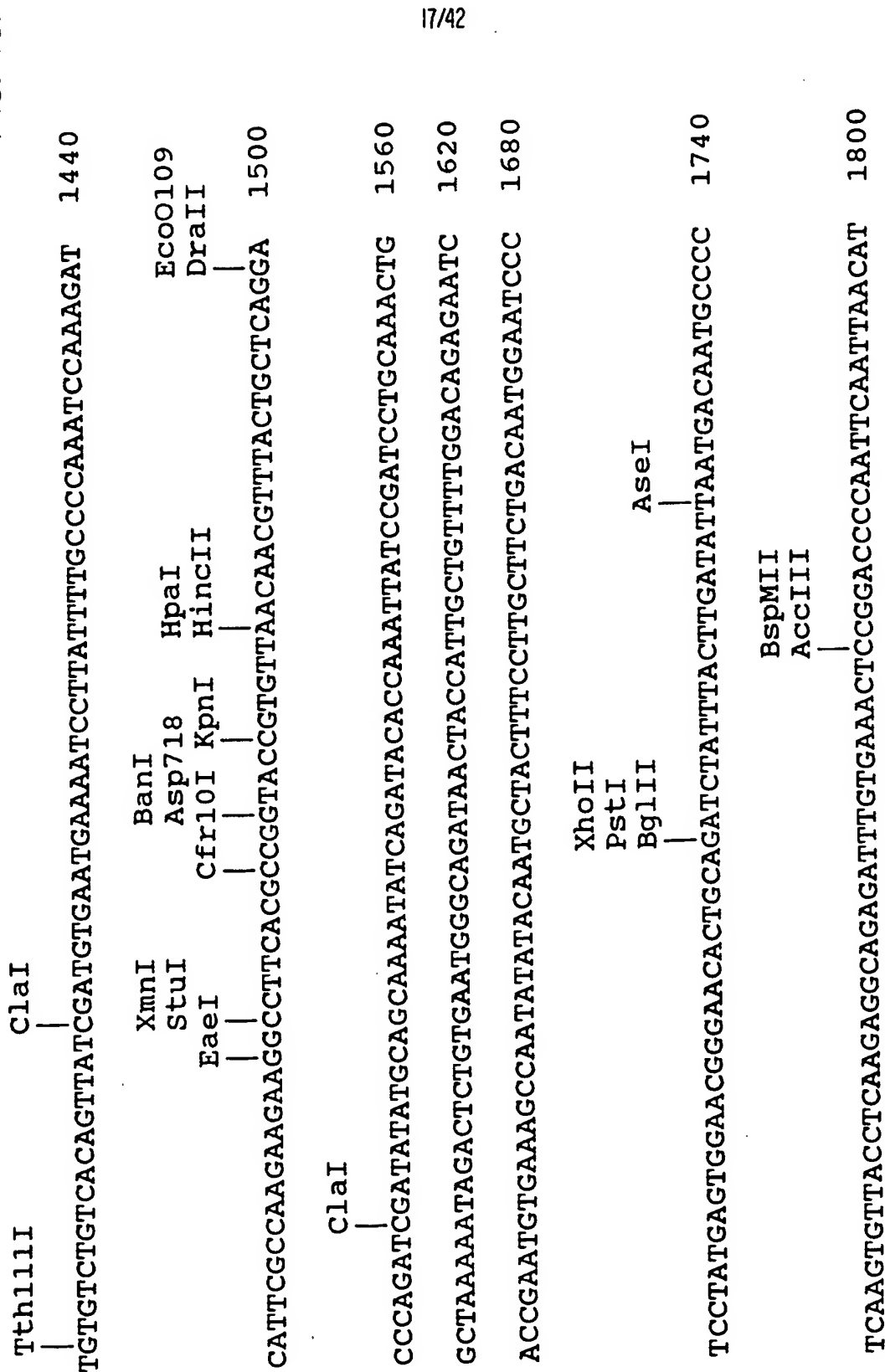


FIG. 4f.

18/42

CACAGCACTTGATTATGACATTGATCCAAATGCTGGACCAATTGCTTTTGATCTTCCTTT 1860
 PflMI
 GTCTCCAGTGACTATTAGAGAAATTGGACCATCACTCGGCTTAATGGTGATTTGCTCA 1920
 CelII
 GCTTAACTTAAAGATAAAATTTCTTGAGGCCGGGATCTACGAAGTTCCAATCATAATCAC 1980
 XhoII
 AGATTGGGGTAATCCTCCCAAATCGAATATCTCCATCCTTCGGGTGAAGGTTTGCCAGTG 2040
 Cfr10I
 Bsp1286
 Bani Bani
 TGATTCCAACGGGGACTGCACAGATGTGGATCGAATTGTGGAGCAGGGCTGGGCACCGG 2100
 HaeII
 BbeI
 NarI
 AhaII
 | |

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FIG. 4g.

CGCCATCATGCCATCCTGCTTTGGCATCATCATCCTGCTCATTTCTGTTCTGATGTTTCGT 2160
GGTATGGATGAAACGCCCGGATAAAGAACGCCAGGCCCAAACAACCTTTTAATTGATCCAGA 2220

AGATGATGTAAGAGATAATATTTTAAATATGATGAAGAAGGTGGAGGAGAAGAACCA 2280

GGACTACGATTGAGCCAGCTCCAGCAGCCTGTATACGGTAGAGCCAGATGCCATCAAGCC 2340

AGTTGGAATCCGACGGTTGGATGAGAGGCCCATCCATGCGGAGCCCCCAGTACCCGGTTCCG 2400

ATCTGCAGCCCCACACCCAGGGGACATCGGGGACTTCATTAAATGAGGGCCCTTAAAGCTGC 2460

TGACAAACGATCCCACCGCTCCGCCCTACGACTCCCTCTTAGTCTTTGACTATGAAGGCAG 2520

TGGCTCCACGGCGGTCCTTGAGCTCCCTTAATTCTCCAGTAGTGAGGTGAGCAGGA 2580

DraI

SspI AhaIII

Bsp1286
BanII

EaeI

EcoO109
EaeI
DraII

PstI

AseI

SstI
SacIEcoO109
Eco52I
EagI
DraII

HgiAI

Bsp1286

BanII

SUBSTITUTE SHEET

FIG. 4h.

20/42

Bsp1286
 BanII
 ApaI
 EcoO109
 DraII
 EcoO109
 EaeI
 DraII
 NSpHI
 AflIII
 CTATGACTATCTGAACGACTGGGGCCCGCTTCAAGAACTCGCTGACATGTACGGTGG 2640
 AGGTGATGACTGAACCTTCAGGGTGAACCTTGCTTTTGGACAAGTACAAACAATTGCAACT 2700
 BsmI
 AccI
 GATATTCCCAAAGCATTTCAGAAAGCTAGGCTTTAACTTTGTAGTCTACTAGCACAGTGC 2760
 AlwNI
 Bsp1286
 BanII
 TTGCTGGAGGCTTTGGCAGAGGCTGCAAACCAATTGGGGCTCAGAGGGAATATCGGTGAT 2820
 SstI
 SacI
 HgiAI
 Bsp1286

21/42

FIG. 4i.

BanII
|
CCAATACTGTTGGAAAACACTGAGCTCAGTTACACTTGAATTTTACGTACAGAAGCAC 2880
TGGGATTTTATGTGCCTTTTGTACCTTTTTCAGATTGGAATTAGTTTATGTTTAAAGGC 2940
SSpI
|
TTTAATGCTACTGATTTCTGAAATGATAAGTAAAGACAAAATATTTTGTGGTGGGAGCA 3000
GTAAGTTAAACCATGATATGCTTCGACACGCTTTTGTGTACATCGCATTTGCTTTTATTAA 3060
StyI
|
AAATATGGAATTAAACAGACAAACCAACCACCTCATGGAGCAATTTTATTACCTTGGGGGC 3120
BstXI
|
TGAGACCATGAGATTGGAAAATGTACATTATTCTAGTTTTAGACTTTAGTTTCTTGTTT 3180
PvuII
|
TGTTTTTTTTTCCACTAAATCTTAAACTTACGCAGCTGGTTGCAATAAAGGAGTT 3240
XmnI
|
TTCATATCACCAATTGTAGCAAAATTGAATTTTTCATAAACTAGAAATGTTAGACACAT 3300
TTTGGTCTTAATCCAATGTACACTTTTTTATTATTTACTGTATTTTTTCCACTTCACTGTAAAA 3360
ATGGTATGTGTACATAATGTTTTTATTGGCCATAGTCTATGGAGAAAGTGCAGAAACTTCAGA 3420

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FIG. 4j.

22/42

NspHI			
AflIII		NspHI	
ACATGTGTATGTATTATTGGACTATGGATTCAGGTTTTTTGGCATGTTTATATCTTTTCGT			3480
TATGGATAAAGTATTACAAAACAAAGTGACATTTGATTCAATTGTTGAGCTGTAGTTAG			3540
AATACTCAATTTTTTAATTTTTTTTAAATTTTTTTTATTTTTTCTCTTTTTTGTGTTGGGG			3600
AGGGAGAAAAGTTCTTAGCACAAATGTTTTTACATAAATTGTACCACAAAAACAAAAAA			3660
	BstEII	PstI	
AAAGGAAAGACAAAGAAATGAAAGGGGTGACCTGACACTGGTGGTACTACTGCAGTGTGTG			3720
	DraI	DraI	
AhaIII		AhaIII	
TTTTTAAAAAAATGAAAAAAGCTTTTAAACTGGAGAGACTTCTGACACACAGCT			3780
TTGCCTCTGTATTGTGTACCAGAAATATAATGATACACCTCTGACCCAGCGTTCTGAAT			3840
AAAATGCTAATTTTGGAAAAAATAAAAAA			3875

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FIG. 4k.

P-cadherin restriction map

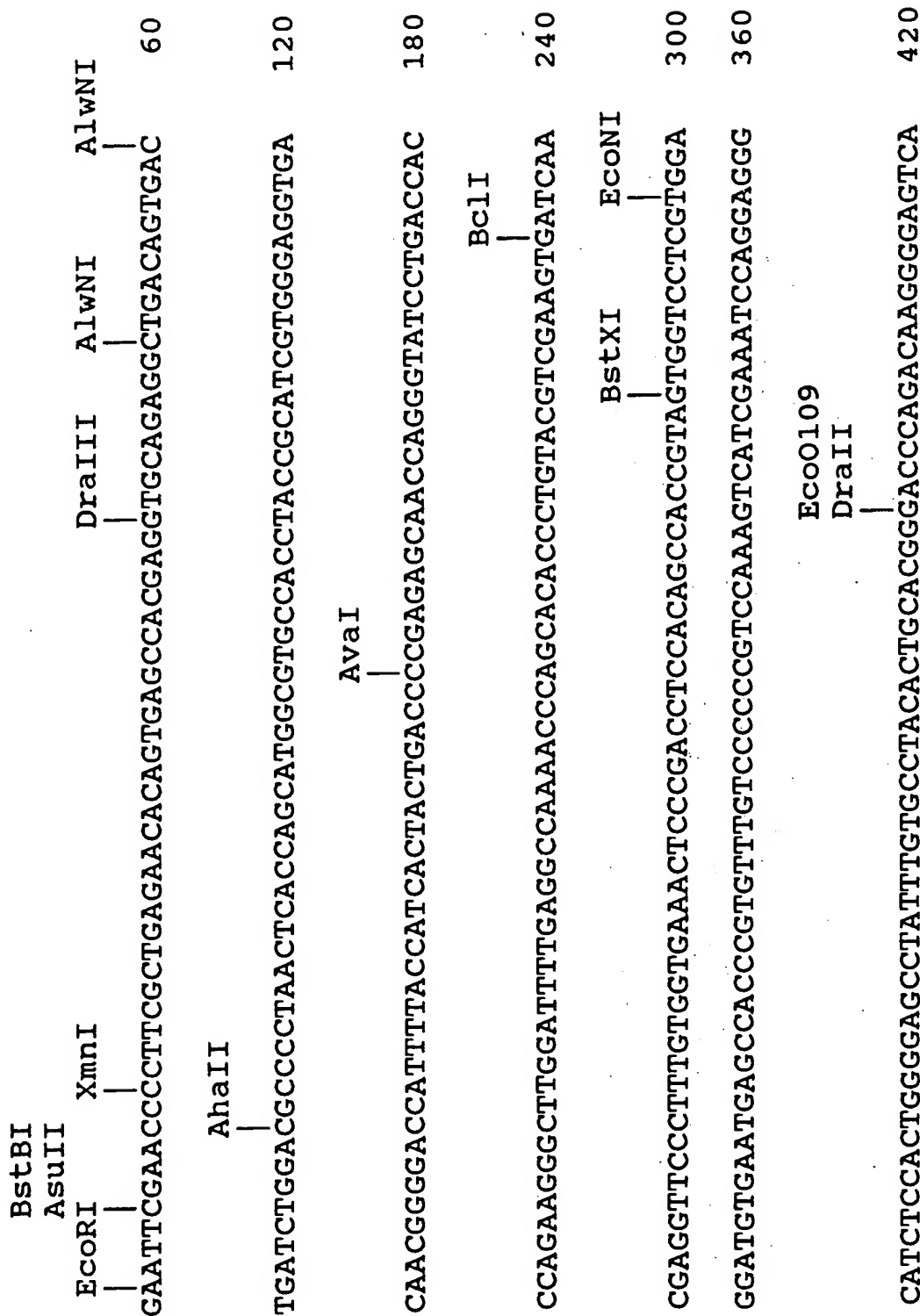


FIG. 4I.

24/42

NheI
 BstXI
 PflMI
 480
 GAAGATCAGTTACCATCCTGAGAGACCCAGCAGGCTGGCTAGCGATGGACCCAGACAG
 TGGACAAGTCACTGCCCGCAGGGGTCTTTGGACCGTGAGGATGAGCAGTTTGTGAGAAACAA 540
 Bsp1286
 Bsp1286
 BanII
 600
 CATCTACGAAATCATGGTCTTTGGCCACAGATGATGGAGCCCTCCACCACTGGCACAGG
 Eco0109
 DraII
 Bsp1286
 Bsp1286
 BanII
 660
 GACCCCTCCTGCTAACACTGATGGACATCAATGACCACGGTCCGGTCCCCCGAGCCCCGTCA
 Bsp1286
 720
 GATCACCATCTGCAACCAAGCCCTGTGCCCCCAGGTGCTAAACATCACAGACAAGGACTT
 EaeI
 AatII
 AhaII
 780
 GTCCCCCACA CTGCCCCCTTTCCAGGCCCAACTCACACATGACTCGGACGTCTATTGGAC
 HincII
 XmnI
 840
 AGCAGAAAGTCAACGAGAAAGGAGAGACGCAGTAGCCCTTGTCCTGGAAGAAGTTCCTAAAGCA

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FIG. 4m.

25/42

HgiAI
 Bsp1286
 ApaLI
 AGGCGAATACGATGTGCACCTTTCCCTGTCCGACCACGGCAACAAGGAACAGCTGACAGT 900
 PvuII
 BclI
 DraIII
 BstEII
 BspMI
 EcoO109
 DraII
 GATCAGAGCCACCGTGTGTGACTGCCACGGCAACATGCTGACCTGCCGGGACCCCTGGAC 960
 GTGGGGTTTCCTCCTCCCATCCTGGGTGCTGCCCTGGCTCTGCTGCTCCTTCTGCTGGT 1020
 HgiAI
 Bsp1286
 XmnI
 GCTCCTATTCTTGGTGAGAAAGAAACGGAAGATCAAGGAACCCCTTCTCCTCCAGAAGA 1080
 Tth111I
 TGATACCCGTGACAAACGTCTTCTACTACGGCGAAGAGGGGGGTGGCCGAGGAGGACCAGGA 1140
 SauI
 Eco81I
 Bsu36I
 FaeI
 CTATGACATCACCAGCTCCACCGGGGTCTGGAGGCCCGGCCCTGAGGTGTTCTCCGCAA 1200

FIG. 4n.

26/42

Bani

CGATGTGGCACCATCCTTCATCCCCACCCCATGTACCGTCTCGGCCAGCCAACCCAGA 1260

1320 TGAATCGGCAACTTCATCATTTGAGAACCTGAAGGCAGCCAAACACAGACCCACGGCCCC

1380 G C C C T A C G A C T C C C T G T T G G T G T T C G A C T A T G A G G C A G T G G C T C C G A T G C C G C C T C T C T

It's

SacI

HqIAI

Bsp1286

BanII

GAGCTCGCTCACCTCCTCAACCTCTGACCAGGACCAAGACTACAACCTATCTGAATGAGTG 1440

NSP-HI

AflIII

GGGCAGCGCTTCAAGAAGCTGGCGGACATGTACGGGGGCCAGGACGACTAGGACTC 1500

Post I

Styl

styI

Bali

CCTAAACGCCGGCTGCAGCAGCGTCTCCAAGGGTCACTATCCCCACGTTGGCCAAGGA 1560

StuI

EaeI

CTTTGCAGCCTTGTTGAGAAATTGGCCTTAGCAACTTGGAGGGAAGGCCTCGAAACTGAC 1620

FIG. 40.

27/42

BspMI
 |
 CTCAAAGGGCAGGTCTCTATGCCCTTTCAGAACGGAGGAACGTGGGCAGTTTGATTTCAA 1680

 HgiAI
 Bsp1286 EcoNI
 |
 CAGTGAGCACCTCTTAGCCCTAAGCCAGGGCTGCTCAATTCTGGGAGTCTCCTCGCTACC 1740

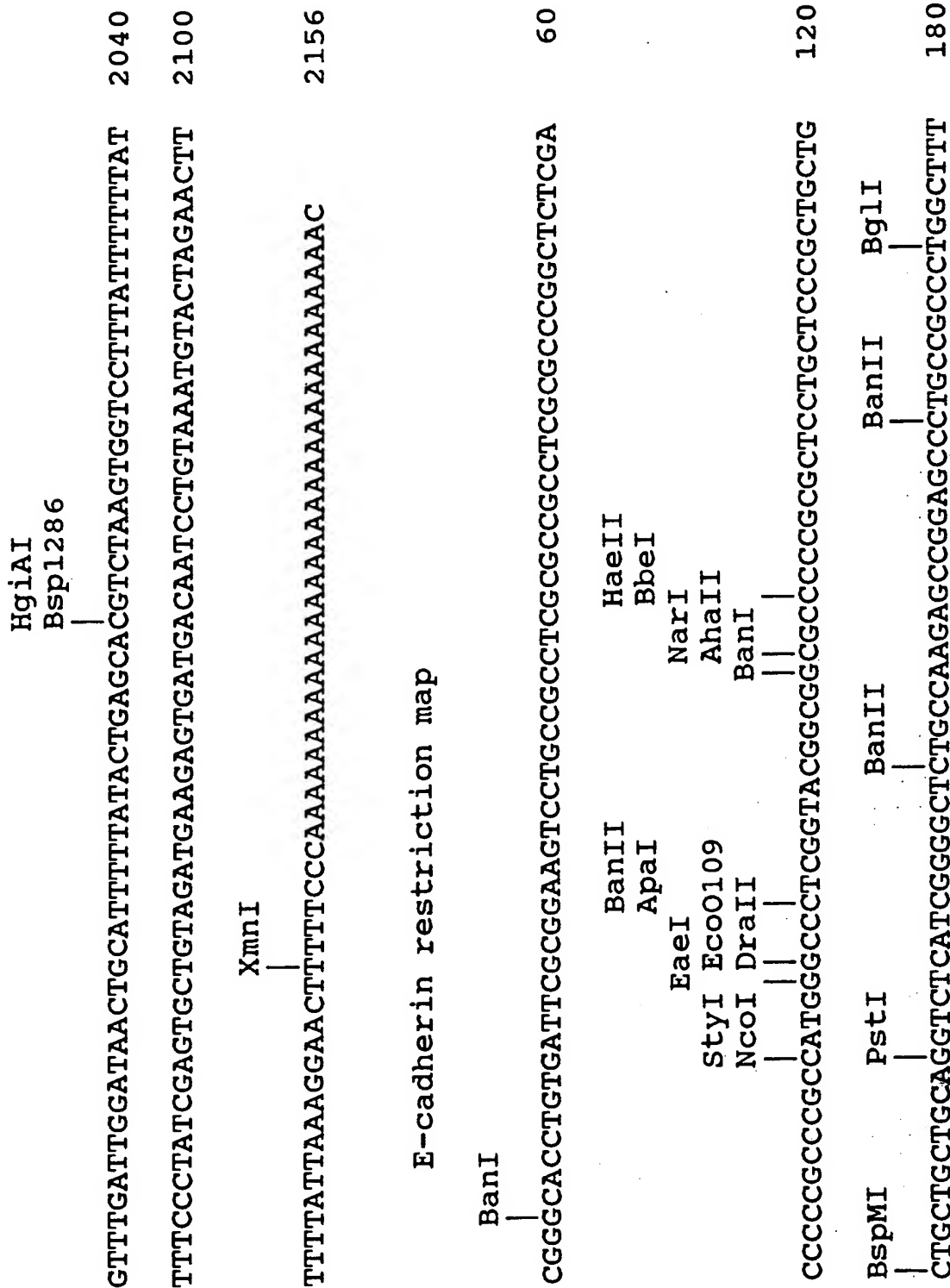
 EcoO109
 DraII
 Eco47III
 CelII HaeII
 | | |
 ATAAATGCTCAGCGCTGGGTCCTGGGTTTGTGACTGACTCTGACTTTCCTCCATGATGGCTT 1800

 StuI
 EaeI
 | |
 TTGCTCTGGAATGGACCCCTTCTCCTTAGTAACAGGCCCTCTTACCACAATCTTCGTTTTT 1860

 EcoO109 HaeII
 BspMI DraII PflMI Eco47III
 | | | |
 TTTTTTTAATGCTGTTTTTCAAAAAGTGAGAGGCAGGTCCTCAACCCCTGGAGCGCT 1920

 Bsp1286 NsiI
 |
 CCAGAAGCCCAGGCGTGCCCTCATGCAATTTCTGTGGTCTCTTGCCCCCAGACCTCCT 1980

FIG. 4p.



E-cadherin restriction map

FIG. 4q.

HaeII AflIII
 |
 GCGGCTGACAGCTACACGTTACCGTGCCCCGGCGACACTTGGAGAGAGGCCGTGTCCCTG 240

styI
 AccI
 Cfr10I AvrII
 | |
 GGCAGGTGAGTTTGAAGGATGCACCGGTCTACCTAGGACAGCCCTATGTTTCTGATGAC 300

ACCCGATTCAAAGTGGGCACAGATGGTGTGATTACAGTCAAGCGCCCTCTACAACTTCAT 360

AAACCAGAGATAAGTTTCTTGTCCTGCTGGACTCCAGCCGCAGGAAGCTCTCCACC 420

BspHI
 |
 AGAGTTAGGCTGAAGGCAGCGACGCCACCACCACCACCATCATGATGCTCCCTCTAAA 480

HgiAI
 |
 ACCCAGACAGAGGTGCTCACATTTCCCAGTTCCCAGCATGGACTCAGAAAGACAGAAAGAGA 540

PvuII EaeI
 | |
 GACTGGGTTATCCCTCCTATCAGCTGCCCGGAAACGAGAAAGGCCCATTTCCCTAAAAAC 600

BalI
 |
 CTGGTTCAGATCAAGTCTAACAGGGACAAAGAAATCAAGTTTCTACAGCATCCTGGC 660

29/42

SUBSTITUTE SHEET

StyI

FIG. 4r.

720

CAAGGAGCTGACGCACCTCCTGTTGGTGTGTTATTATTGAAGAGAAACAGGATGGCTG

780

AAGGTGACTGAGCCTCTGGATAGAGAAACAAATTGCTAAGTACATTCTCTACTCTCATGCC

BsmI

BclI

840

GTATCTTCTAATGGGAATGCGGTTGAAGACCCCAATGGAGATCGTGATCACGGTGACAGAT

AvaI

XhoII

StyI

BamI

900

CAGAAATGACAACAAGCCCGAGTTCACCCAGGCAGTCTTCCAAGGATCTGTCAACGGAAGGT

BamI BspMI

960

GCCCTTCCAGGCACCTCTGTGATGCAGGTGACAGCCACAGATGCCGGATGATGTGAAT

1020

ACCTACAACGCTGCCATCGCTTACAGCATCCTCACACAAGACCCCTCCTGCCTAGCAGC

HgiAI

BstXI

1080

ATGATGTTCACTATCAACAAGGACACAGGAGTCAATCAGCGTGCTCACCACTGGGCTGGAC

StyI

BspMI

1140

CGAGAGGGTGTCCCAATGTACACCTTGGTGGTTCAGGCTGCTGACCTGCAAGGCCAAGGC

30/42

SUBSTITUTE SHEET

32/42

FIG. 4t.

ACATATATGGAACAGAGGATAACGTATCGGATTGGAGGGATGCTGCCGGTTGGCTGGAG 1680
 BanI
 PflMI
 AlwNI
 AvaI CeuII
 GTTAATCCAGAAATCTGGTGCCATTTTCACTCGGGCTGAGCTGGACAGAGAGGATTTTGAG 1740
 HgiAI
 CACGTGAAGAATAGCACGTATGAAGCCCTCATATAGCCATTGACTTCGGTTCCTCCAGTT 1800
 GCTACTGGAACGGGAACTCTTCTACTGGTCCTCTCTGATGTGAATGACAATGGCCCCATT 1860
 CCAGAACCTCGAAATATGGACTTCTGCCCAGAAAAACCCACAGCCCTCATGTCTCATCAACATC 1920
 XhoII
 BglII
 ATTGATCCAGATCTTCCCCCAACACATCTCCCTTCACAGCAGAACTAACACACGGCGCA 1980
 HincII
 AGTGTCAACTGGACCATCGAGTACAATGACCCAGCTCGTGAACTCTCTAATTTTGAAGCCA 2040
 AAGAAAACTTAGAGTTGGGTGACTACAAAATAAATCTCAAGCTCACAGATAACCAGAAC 2100
 BstEII
 PvuII
 HincII
 AAGGACCAGGTGACCACCCCTATATGTGTGTTGTGTGCGACTGCCGAAGGTGTCGTCAACAGC 2160

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FIG. 4w.

35/42

DraI AhaIII 	ACTTGTCTCATTTTAAAGGAAGGTAGGGCTAAACTACCCCTATTGTGTTGTGTGTGT	3120
	GTGTGTGTATGTGTAATTATTTTAAATTGTGTCTCTTTTCTCCCTATCAGTGCACCTGGT	3180
	GTCCCGTGTCTAATAACCACTCTTAACTCCTTCTGAACTTACATTGCCCTCAGACAGGAG	3240
	EcoNI 	
BanII ApaI EcoO109 DraII EaeI PstI 	TTCCTCTGCTGCAGAAATTATTGGGCCCTTTCAGGATAAGAGACTTGGTCTTAGTTTGATG	3300
	GTAAGTGTGACTGGGTATTATGGACTCGTAAGGACTTTAGTGGTCTCCTTTTATTATTTCC	3360
	TAAGTACATAAAATTGAAATTTCATATCCATCCACTGACTTGTCTGCAATTAAGTGTGTTTG	3420
AatII AhaII 	TCATGTGGACGTCATTATTGGGGCTACTTTGGTTCTGAAACAAGGAGCATTGACCAGAAAAG	3480
BanI 	GTGGTGAAFTTTCAGGTGCCCACTCAACTTCTAATGTTCACTTATCACTCAAAACAGAAGAG	3540

SUBSTITUTE SHEET

36/42

FIG. 4x.

	PstI	PstI	
TGATCTATTCTGACGTTTAGCGTAGTGCCCTGCCAGTGCTGCAGCCAAAGATTGAAGCGGA			3600
	StyI		
TTGTCAAAGCCAAGGCAACATGAATAATGGACTTGGAGGTGGCAGCGGGATGGGTCTAT			3660
TGAGCCCTGGCGTTT TAGCAAAC TGATGCTGAGGATAACTGAGGTGGCTCTACCTCTAGTC			3720
		SauI	
		Eco81I	
		NruI	
		Bsu36I	
CTGAAAAATTCTGAAGAAATGGAAGAAATCCCGACAAGTGTGCTCCTATCGCGATCCTTAGGTC			3780
	SauI		
	Eco81I		
	Bsu36I		
ACAGTTTGTAACCTGAGGCCAAGAAATCCCGAGGTGCCCTGCTTTTGTAAATGTCTACCGAAA		BanI	
		AccI	
			3840
	BanI		
ATGCAGCCTGATCTGGACTCAGGTGCCCCCAATTCTAAGTGTGCATAGAAAACTGACAATA		SspI	
			3900
	SauI		
	Eco81I		
	Bsu36I		
TTAGGAAATTCTTTTCCCCCCTTAGGAGCAGGAAGAAAAATATGACCCCTAAAGGGTTTGTG			3960

SUBSTITUTE SHEET

37/42

DraI
AhaIII
|
GCAAAGGGAAGGTGGGAGAGCCTTTGACTTGGAATTTTTTAAATTGAAATGTGAACTTC 4020

StyI
NcoI
|
AAGGAACTTTTGACAACCATGGGAAATAATTTTATCTTAAATTGCTTTACTGCTGTCAG 4080

PvuII
|
CTGTTTTTCAAGAAAAAAATCATCCCTGCAATCACTTCTTGGAATTGTCTTGATTT 4140

DraI
AhaII
|
TTCAGCAATTTAAACTCTAATTTAGTCCTGTATAGAGAAATGTTAATGTAGTTTGTAGTGT 4200

ATATGTGTGTGGGTACGGATAATTTTGTATTTTCTTTAGGCTCTGGAAAAGGAAAACAATT 4260

SspI
|
TAAGCTGCGAAAATTCCTTAAATATTCATTTTATAAAATTTTATTAAGAAATTTTGTATAA 4320

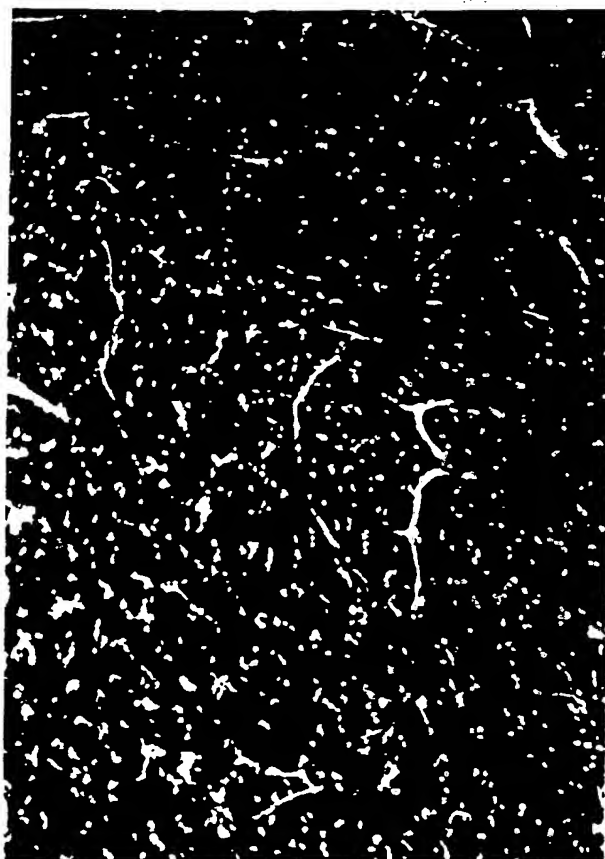
AAAAAAAAAAAAA 4333

FIG. 4y.

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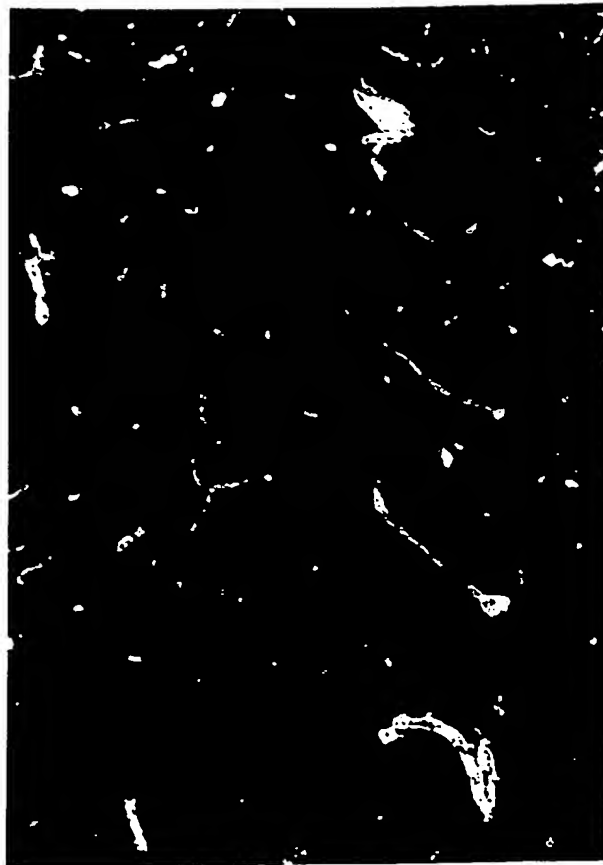
38/42

FIG. 5.



39/42

FIG. 6.



40/42

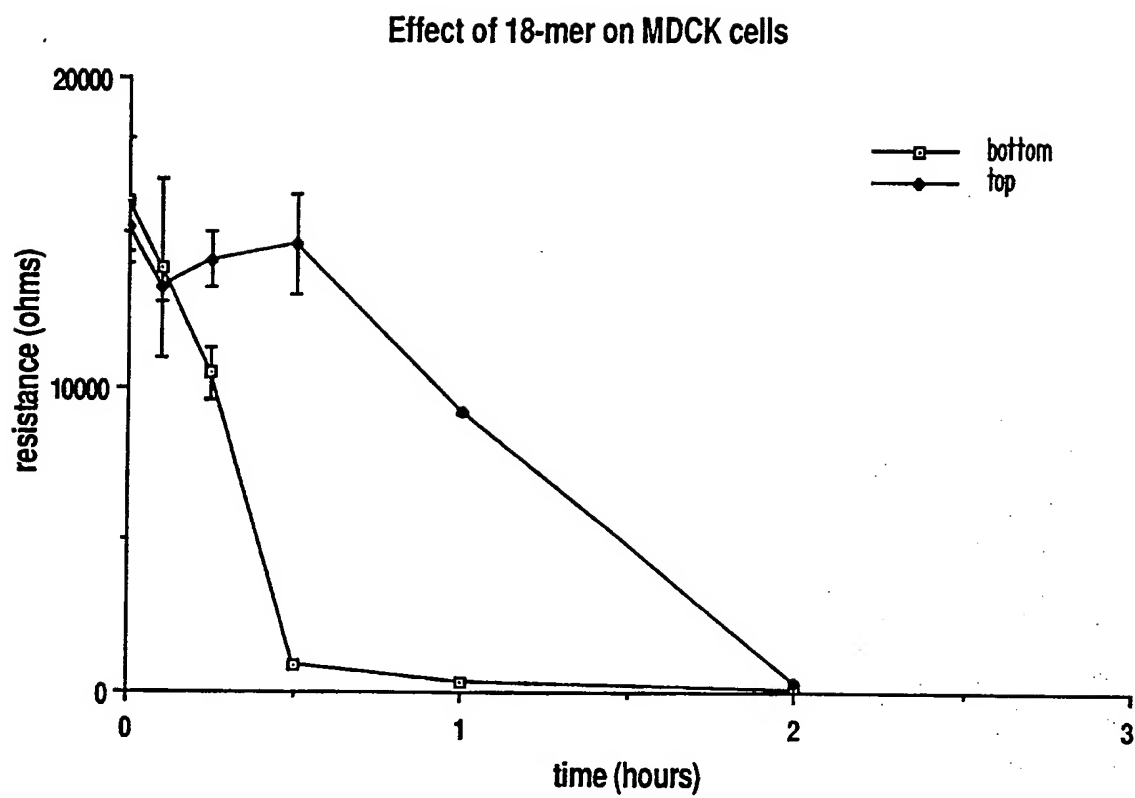


FIG. 7.

41/42

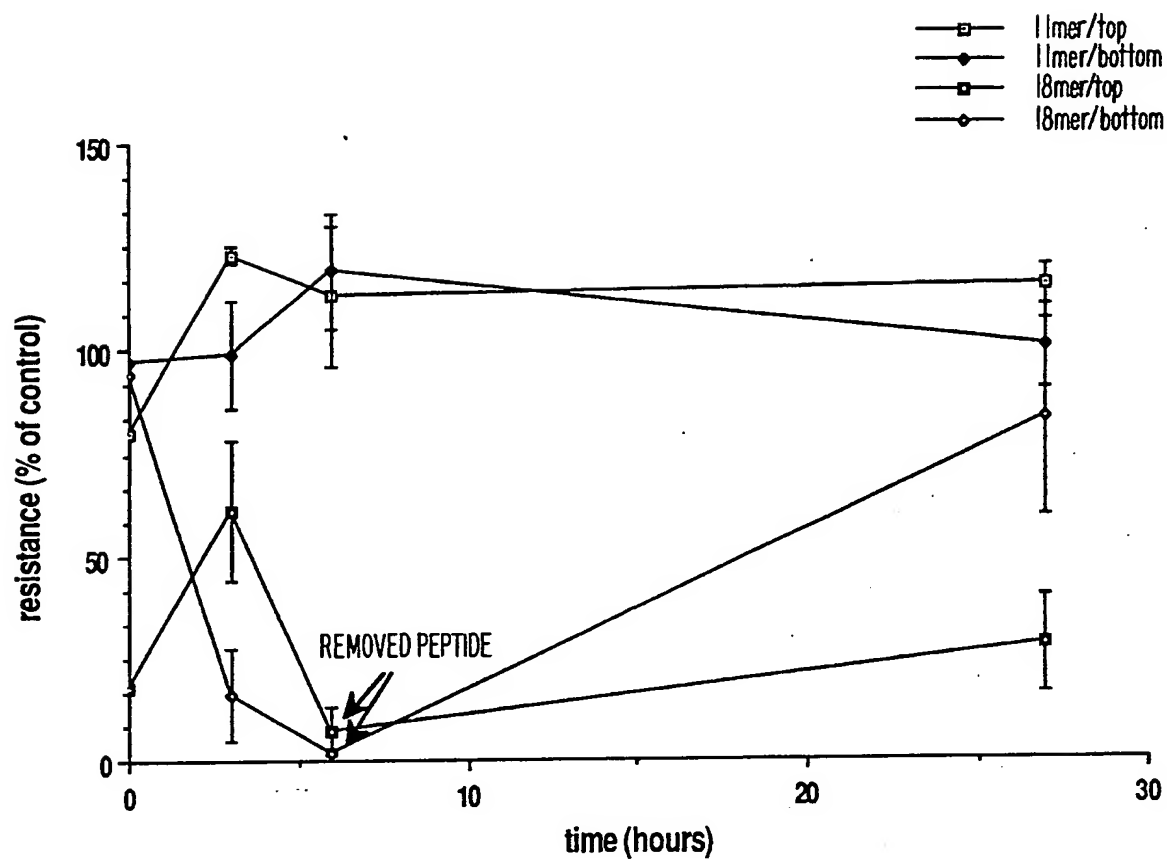


FIG. 8.

42/42

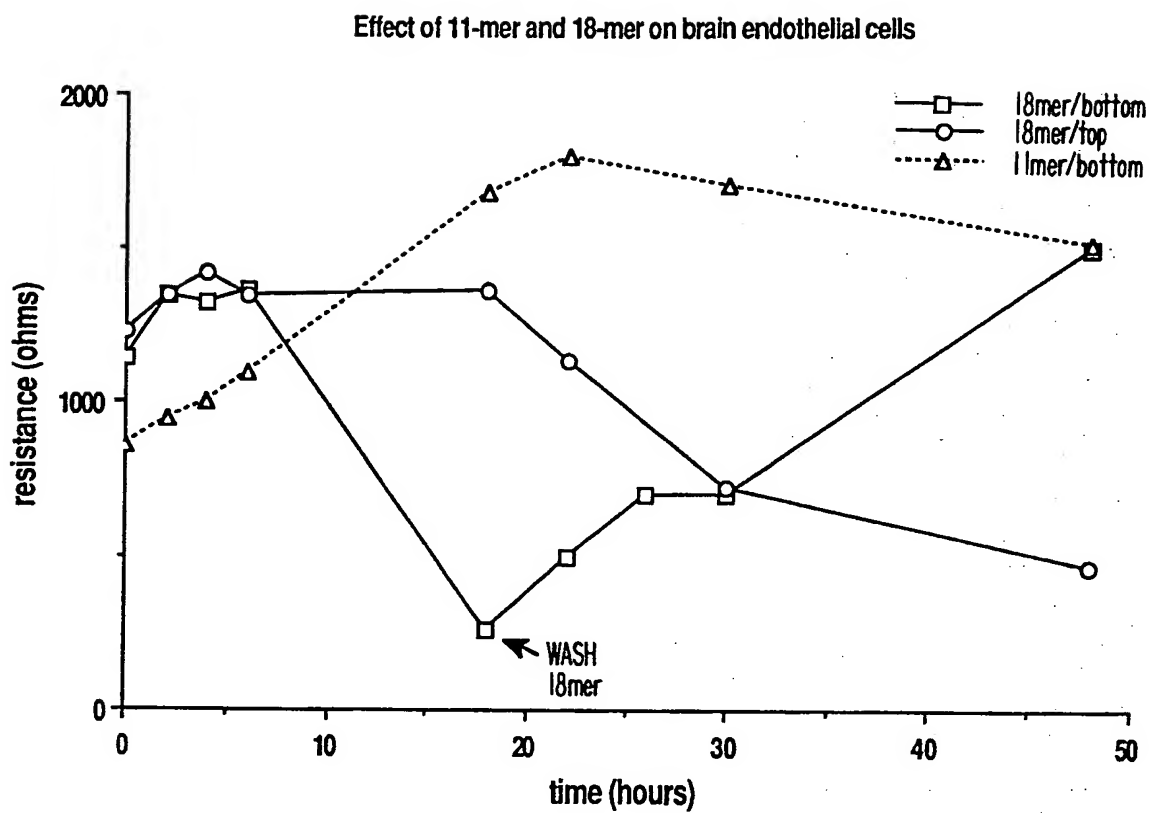
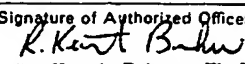


FIG. 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05105

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(5): A61K 37/02, 39/00; C07K 7/08, 7/10, 13/00, 15/00, 15/28		
U.S. Cl.: 530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387; 514/12, 13; 424/85.8, 85.91		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System ¹	Classification Symbols	
	530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387	
	514/12, 13	
U.S. Cl.	424/85.8, 85.91	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
Data bases: Dialog (Files; Medline, Biosis, Chemical Abstracts, World Patents Index) Automated Patent Searching (1975-1990)		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ^{1*}		
Category [*]	Citation of Document, ^{1*} with indication, where appropriate, of the relevant passages ^{1*}	Relevant to Claim No. ^{1*}
X Y	The EMBO Journal, Volume 4, No. 13A, issued December 1985, Vestweber et al., "Identification of a Putative Cell Adhesion Domain of Uvomorulin," pp. 3393-3398, See the Abstract and Discussion.	1-6,14-21,23-27 & 35-42 1-65
Y	Development, Volume 102, issued April 1988, M. Takeichi, "The Cadherins: Cell-cell Adhesion Molecules controlling Animal Morphogenesis," pp. 639-655 see the Summary and pages 643, 645 and 651.	1-65
X Y	The Journal of Cell Biology, Volume 107, issued October 1988, B. Gumbiner et al., "The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex," pp. 1575-1587 see the Abstract.	1-6,14-21,23-27, 35-42 1-6,14-27,35-47, 55-65
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ^{1*}</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²	
21 November 1990	04 FEB 1991	
International Searching Authority ¹	Signature of Authorized Officer ^{2*}	
ISA/US	 R. Keith Baker, Ph.D.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
Y	The EMBO Journal, Volume 6, No. 12, issued 1987, M. Ringwald et al., "The Structure of Cell Adhesion Molecule Uvomorulin Insights into the Molecular Mechanism of Ca ²⁺ -dependent Cell Adhesion," pp3347-3353, see pages 3647-3648.	1-13,22-34,43-54 and 63-65
Y	US, A, 4.671,958 (Rodwell et al.) 09 June 1987, see the Abstract and Column 7.	43-47 and 55-65
Y,P	Development Biology, Volume 139, No. 1, issued May 1990, O.W. Blaschuk et al., "Identification of a Cadherin Cell Adhesion Recognition Sequence," pp227-229, see the entire Document.	1-65

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter¹ not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Attachment To PCT/ISA/210
Observations Where Unity Of Invention Is Lacking

Group I, claims 1-13 and 22-34, drawn to a composition for opening tight junctions and a method of use, classified in classes 530 and 514, subclasses 324, 326, 350 and 12 and 13, respectively.

Group II, claims 14-21 - 35-42, drawn to antibodies for opening tight junctions and methods of use, classified in classes 530 and 424, subclasses 387 and 85.8, respectively.

Group III, claims 43-54 and 63-65, drawn to a conjugates of a drug and a cell adhesion inhibitor, classified in class 530, subclasses 402, 409, and 345.

Group IV, claims 55-62, drawn to a conjugate of a drug and an antibody, classified in classes 530 and 424, subclasses 389, 390, 391 and 85.91, respectively.

Attachment To PCT/ISA/210

Detailed Reasons For Holding Lack Of Unity Of Invention:

PCT Rule 13.2 permits claims to "a" (one) product, "a" (one) method of making and "a" (one) method of using said product. The invention as set forth in Group I constitutes a combination of a product and a method of use. Groups II, III and IV one drawn to products that are distinct from that of Group I. Each of the products have a different structure and are distinct compositions as evidenced by their separate classification.

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